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Dermal Absorption of (14)C-Labeled 4,4′-Methylenedianiline (4,4′-MDA) in Rats, Guinea Pigs, and Monkeys

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16. Abstract (Limit: 200 words)
To help determine workplace exposure to 4,4'-MDA, studies were performed in male rats, g. pigs and monkeys treated topically with a low (2 mg/kg) or high (20 mg/kg) dose of ^{14}C-4,4'-MDA. Conditions of treatment (dosage, dose regimens, and occlusion) were assessed. The disposition of 4,4'-MDA was also examined after i.v. dosing. In rats, 43 and 10% of the low dose was recovered in urine and feces during a 96 h period; 2% remained in tissues and skin washing removed 25% of dose. The remainder (26%) was recovered by skin extraction and solubilization. The percent of dose absorbed decreased by increasing the dose, but the total amount absorbed (~0.25 mg/rat) was similar after both doses. In g. pigs, 10 and 18% of the low dose was excreted in urine and feces; 1% was recovered in tissue, 41% in the skin wash and 29% from the application area. The percent of dose absorbed decreased following the high dose, but the amounts absorbed (in mg/animal) doubled. In monkeys, 19 and 2% of the low dose was eliminated in urine and feces during 168 h and 47% was recovered in skin wash. Under similar conditions (application of low dose for 24 h followed by excreta collection for 96 h) absorption was comparable in g. pigs and monkeys (~18%) and higher in rats (~43%). When applied 4,4'-MDA was washed immediately with soap/water or acetone/water neither washing method was capable of removing all the applied material from the skin. Higher recoveries were obtained by washing with soap/water; in addition, acetone facilitated absorption. Since the unrecovered material represents amounts associated with the skin available for later absorption, the extent of dermal absorption should be considered higher than calculated from excretory and tissue distribution data only.

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DERMAL ABSORPTION OF ^{14}C-LABELED 4,4'-METHYLENEDIANILINE (4,4'-MDA) IN RATS, GUINEA PIGS, AND MONKEYS

by

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PREFACE

This report includes the results of studies conducted to assess the percutaneous absorption of 4,4'-methylenedianiline (4,4'-MDA) and to examine the effect of several factors on the dermal penetration process. These studies were conducted under MRI Project No. 8501-A, Work Assignment No. 21, for EPA's Office of Toxic Substances (EPA Prime Contract No. 68-02-3938). These studies were performed by Monaem El-hawari (Study Director), Maxine Stoltz, Patricia Alm, and Diane Czarnecki with assistance from Edward Williams, Jack Holcomb, Cristin Mansfield, and Kathy Howe. This report incorporates changes in response to reviewers' comments on the draft final report dated June 28, 1985. The final report was submitted on December 30, 1985.

MIDWEST RESEARCH INSTITUTE

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QUALITY ASSURANCE STATEMENT

DERMAL ABSORPTION OF ^{14}C-LABELED 4,4'-METHYLENEDIANILINE (4,4'-MDA) IN RATS, GUINEA PIGS, AND MONKEYS

This study was inspected by the Quality Assurance Unit as follows:

Phase	Date
Guinea pig quarantine	5/23/84
Rat receipt and quarantine	5/23/84
Animal quarters environmental check	5/24/84
Guinea pig, rat quarantine	6/28/84
Tissue oxidizer operation	7/26/84
Rat, dermal application	8/16/84
Monkey, environmental conditions	8/29/84
Monkey, dermal application	10/10/84
Guinea pig, i.v., dermal application	10/15/84
Guinea pig, dermal application	10/29/84
Monkey, urine, feces collection	12/3/84
Monkey, dermal, i.v. dosing	1/16/85
Data audit	3/12/85
Final report review	6/25/85

Reports were submitted to the study director and management on June 4, 1984, November 27, 1984, and March 12, 1985. This study was done in compliance with the EPA Good Laboratory Practice Standards of November 28, 1983 (Federal Register 48:53922-53944). The methods described were the methods followed and the data presented accurately represent the data generated during the study.

The final report and all records are stored in the MRI Archives.

Eugene G. Podrebarac

Manager, Quality Assurance Unit

EXECUTIVE SUMMARY

Dermal absorption is a prevalent route of exposure for many industrial chemicals. To assist in determining workplace exposure to 4,4'-methylenedianiline (4,4'-MDA), an aromatic amine with known toxic and carcinogenic properties, studies were performed in male Fischer 344 rats, Hartley guinea pigs, and Rhesus monkeys treated topically with ^{14}C-labeled 4,4'-MDA. Conditions of treatment including dosage, concentration, dose regimens, and occlusion were assessed. The studies in rats and guinea pigs were performed at two dose levels: a low dose (~ 2 mg/kg) and a high dose (~ 20 mg/kg). The monkeys were treated with the low dose only. The disposition of 4,4'-MDA was also examined following intravenous (i.v.) administration of the low dose to the three species. Excreta from rats and guinea pigs were collected for 96 h, then the animals were sacrificed for tissue sampling. The studies in monkeys were extended to 168 h and only excreta were sampled.

During 96 h after continuous dermal application of the low dose to rats, 43% of dose was recovered in urine, 10% in feces, and 2% in tissues. Only 25% of dose was removed by washing the skin with soap and water. The remainder (26%) was recovered by skin extraction (methanol) and solubilizing (perchloric acid and hydrogen peroxide). In rats treated dermally with the low dose for 24 h then the dose removed, recoveries in excreta, tissue, and the skin application areas were lower. In rats with the application areas nonoccluded, only 7% of the low dose was recovered in excreta and tissue at 6 h, compared to 12% in rats with occluded skin. After continuous application of the high dose, only 4.8 and 1.3% of dose were eliminated in the urine and feces, respectively. About 0.4% was recovered in tissue, 62% in the skin wash and 24% from the application area. Although the percent of dose absorbed decreased by increasing the dose, the total amounts absorbed (0.2-0.25 mg/rat) remained similar after both doses.

In guinea pigs, 10% of the low dose was excreted in urine and 18% in feces during 96 h following continuous dermal application. About 1% was recovered in tissue, 41% in the skin wash and 29% from the application area. Guinea pigs treated dermally with low doses that were washed at 24 h had lower amounts of ^{14}C in excreta, tissue, and skin application areas. In guinea pigs with nonoccluded application areas, recoveries in excreta and tissue (3% in 6 h) were similar to those with occluded skin. After the high dose, recovery in urine, feces, and tissue averaged 3, 4, and 1%, respectively; 70% was recovered in the dose wash and 14% was removed from the application area. Although the percent of dose absorbed decreased following application of the high dose, the amount absorbed (in μg/kg) was doubled. In monkeys, 19 and 2% of the low doses were eliminated in urine and feces, respectively, during a 168 hr period. Under similar conditions of exposure (dermal application of the low dose for 24 h followed by excreta collection for 96 h) comparable absorption was demonstrated in guinea pigs and monkeys (~ 18%) and higher absorption in rats (~ 43%).

In rats treated i.v., most of the dose was eliminated by 24 h; recovery at 96 h averaged 67% in urine and 31% in feces. Most of the i.v. doses were eliminated by 48 h in guinea pigs and monkeys. In guinea pigs, 35% of dose was excreted in urine and 57% in feces during a 96 h period. Monkeys excreted 84% of dose in urine and 10% in feces during a 168 h period. In rats, ^{14}C in blood and tissue averaged 19% of dose at 6 h which declined to 2% by 96 h. The highest levels were demonstrated in liver which was 5-9 times higher than blood. In guinea pigs, ~ 3% of the dose was recovered in blood and tissue at 96 h. The highest content was demonstrated in the spleen (6 times higher than blood) followed by liver. The data suggest considerable elimination through the biliary route especially for guinea pigs.

When 4,4'-MDA applied to the skin of rats, guinea pigs, and monkeys was washed immediately with soap or acetone solutions, as low as 53% of the applied doses was recovered. A post-exposure wash at 6 h, 24 h, and 96 h removed less of the applied material. The studies showed higher and more consistent recoveries when the application areas were washed with soap and water versus acetone and water. However, both washing methods were incapable of removing all the applied material from the skin. Significant amounts of the applied doses remained associated with the skin available for delayed absorption. In addition, acetone facilitated absorption. Since 4,4'-MDA associated with the skin is available for systemic circulation, the extent of dermal absorption in rats, guinea pigs, and monkeys should be considerably higher than calculated from the excretory and tissue distribution data only.

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I. INTRODUCTION

Under the Toxic Substances Control Act (TSCA), a manufacturer or processor must submit to the Environmental Protection Agency a premanufacturing notice (PMN) for every new chemical prior to commercial production. The Agency has 90 days to review the PMN and to take any regulatory actions deemed necessary. As part of this review, the potential workplace exposure and dose associated with the manufacturing and processing of the new chemical must be estimated. The estimate of dose is particularly relevant for risk assessments. Dose, however, is quite difficult to estimate, especially for dermal exposure, which may be the prevalent exposure route in many manufacturing and processing operations. Currently, very little information is available that would allow the prediction or estimation of dermal penetration rates.

The overall objective of this task was to develop dermal penetration data for 4,4'-methylenedianiline (4,4'-MDA, Figure I.), an aromatic amine with known toxic and carcinogenic properties. The specific aims were to select and compare techniques and animal models for measuring dermal penetration rates of this compound. The studies were performed *in vivo* with three animal models: the rat, guinea pig, and monkey. Several experimental conditions were considered and assessed in the study protocol including skin type (rat, guinea pig, and monkey), dosage (low, high), exposure regimen (limited, continuous), occlusion (exposed, covered), and carrier. In addition, initial studies were performed to assess the dermal washing efficiency following application to rats, guinea pigs, and monkeys.

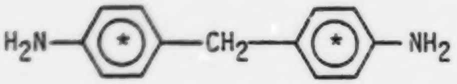

Figure I. 4,4'-Methylenedianiline (4,4'-MDA)
(* The position of the ^{14}C label)

Limited experiments were performed to assess the elimination of 4,4'-MDA following intravenous (i.v.) administration to utilize the data generated in determining the absolute absorption of 4,4'-MDA following dermal application. The utility of this methodology was assessed by comparing the data generated to those resulting from excretory data in urine and feces of animals exposed by dermal application.

The species selected were the rat, a species for which historical data on the toxicity and carcinogenicity of aromatic amines are available and which is used extensively in dermal absorption studies, the guinea pig, and the monkey, species which have skin characteristics that resemble human skin. The studies in rats and guinea pigs were performed at two dose levels, "low" and "high." The monkey studies were carried out with the low dose only.

II. BACKGROUND

The skin is a complex and heterogeneous membrane which provides, in general, a significant resistance to chemical ingress. Nevertheless, the skin is a primary body contact with the environment and the route by which many chemicals enter the body.[25,32,33] Some chemicals applied to the skin have proved to be toxic. Thus, percutaneous absorption is an important route of entry for potentially hazardous and toxic material. The ability to predict dermal penetration would, therefore, be of considerable benefit in the question of cutaneous chemical exposure for which direct testing in humans is not feasible.

The potential for large quantities of toxic chemicals entering the systemic circulation via the dermal route is quite high. Many factors determine the toxic potential of a topically applied chemical.[42] The most obvious is that the chemical be inherently toxic. The systemic availability is then determined by the rate of dermal absorption, concentration of applied dose, surface area covered, and length of exposure. Other factors such as occlusion, hydration, and anatomical site exposed will also affect the absorption.[24,42]

Percutaneous absorption is a process by which a chemical moves sequentially from the surface of the skin through the stratum corneum, epidermis, papillary dermis, and finally into the bloodstream. Resistance to movement through this barrier is not equally distributed among all layers, but several studies have indicated that the stratum corneum is the rate limiting portion.[18] The rate of flux of chemicals from the vehicle into the epidermis and subsequently into the systemic circulation follows a process which can be divided into a lag phase, a rising phase, and a falling phase.[18] The total amounts of chemical absorbed during this process can be calculated from excretion or blood levels data. Although it is theoretically possible for the applied dose to be completely (100%) absorbed, existing data on percutaneous absorption suggest that this is not achieved.[11-15] It is now clear that the skin is a barrier of variable permeability with some compounds being well absorbed. This contrasts with the older notion that skin is a relatively impervious barrier.[32,33]

A definitive structure-activity relationship for percutaneous absorption of many xenobiotics has not yet been established. Although attempts were made to relate chemical structure and chemical partition coefficients to skin penetration, no clear principles are yet available to predict the *in vivo* absorption in man.[5,18] The rate of absorption of topically applied chemicals can vary greatly. The major variables that account for differences in absorption appear to be the chemical concentration, its partition coefficient between the stratum corneum and the vehicle, and its diffusion coefficient into the stratum corneum. Measurements of the rate and extent of absorption of topically applied chemicals in man are difficult since most of these chemicals are potentially toxic. In addition, these studies generally require the application of radioactive tracers.[18] When these studies are conducted, the radioactive chemical, dissolved in a suitable vehicle, is applied to the skin and urine is collected and analyzed for radioactivity. When only urinary excretion is used for measuring percutaneous absorption, correction is made for excretion of the tracer by other than the urinary route. This is done in a separate series of experiments in which the percentage of an intravenously (i.v.) administered dose of the same compound excreted in urine is determined.[42]

Although animal skin is unlikely to have permeability properties identical to that of human skin, animals are used for dermal toxicity and absorption evaluations since many of these studies cannot be performed in humans. Penetration of toxic compounds can also be measured satisfactorily with excised skin in diffusion cells.[8,9,16,17] No one animal will simulate the penetration in humans of all compounds, although the rhesus monkey and miniature pig yielded the best correlation with human penetration.[28,35,38-41,44] Rabbit skin is generally recognized as the most permeable of the commonly used lab animals.[38] Mouse and rat skin also showed high permeability.[42] Although data on guinea pig skin are limited,[1] this species is used for dermal toxicity studies and is considered a sensitive indicator of potential dermal toxicity. Other animal models examined include the hairless dog and the hairless mouse.[29-31]

Several studies have shown that values for even the most permeable skin, such as rabbit or mouse, are often well within an order of magnitude of values for human skin. Thus, it appears that, depending on the compound of interest and the vehicle used, values that are not too dissimilar from those with human skin might be obtained with the skin of a number of animal species. In studies by Bronaugh et al.,[8] several test compounds were ranked in the same order of permeability with all four types of animal skin employed (pig, rat, mouse, and hairless mouse). The animal model of choice, however, may depend on the compound of interest. They suggested that an initial comparison using human skin may be made _in vitro_ before an animal model is selected for routine testing. It should be noted that only a limited amount of human and animal permeability data is available and that generalizations cannot be made.

Laboratory rodents are more convenient than the monkey or the pig for dermal toxicity and absorption studies because of ease in handling and lower cost. The skin of the rat has been considered to be more permeable than human, pig, or monkey skin. However, in several studies with caffeine, butter yellow, and N-acetylcysteine, rat skin appeared to be at least as good a model for human skin as pig skin.[3,4] When rat skin has been found to be more permeable than human skin, the differences have not been large. It was found that the stratum corneum of female rats is as thick as that of human skin. The stratum corneum of male rat back skin is almost twice as thick as that of the female rat and represents a better barrier against absorption.[8] Recently, the hairless mouse has had increasing use because of the reported similarity of its skin to human skin in the absorption of antiinflammatory agents and aliphatic alcohols.[42]

Percutaneous skin absorption can be measured in living animals or humans (_in vivo_) or _in vitro_ by using excised skin in diffusion cells.[7,16,23,27] The studies with excised skin seem feasible since passage through the skin is a passive diffusion process and the primary barrier, the stratum corneum, is composed of nonliving tissue. The selection of whether to measure skin penetration _in vivo_ or by _in vitro_ techniques depends at least on the utility of the data obtained. _In vivo_ studies measure the absorption rate indirectly, e.g., by determining the excretion of a compound from the body, or the blood, or tissue levels of the compound. The resulting data include physiological effects on an absorbed compound such as metabolism, distribution, and excretion.[20,21]

In vitro absorption methods have been used recently in percutaneous absorption studies using excised human skin. In initial studies performed by Franz,[16] the permeability of 12 organic compounds were evaluated and compared to those obtained *in vivo* by Feldman and Maibach.[12-15] Although care was taken to ensure that both conditions of exposure were similar, some differences in dosage and anatomical site of skin were noted. The *in vitro* methods distinguished compounds of low or high permeability, but quantitatively the *in vivo* and *in vitro* data did not agree. Although these differences suggest that *in vitro* methods would not always be a reliable or accurate predictor of percutaneous absorption in living humans, more recent studies[8,9,17] suggest that *in vitro* methods can provide useful preliminary information that can be later expanded using *in vivo* experiments. In addition, *in vitro* methods are useful for studies that separate the percutaneous absorption from other pharmacokinetic factors associated with the uptake of compounds by the topical route. Large numbers of experiments can be performed simultaneously and sampling is performed directly under the surface of the skin. Only *in vitro* methods can be used to obtain permeability data on highly toxic compounds with human skin.

III. MATERIALS AND METHODS

A. Animals

Adult male Fischer 344 rats, Hartley guinea pigs, and rhesus monkeys were used in the studies. The rats and guinea pigs were purchased from Charles River Breeding Laboratories, North Wilmington, Massachusetts. The monkeys were obtained from Hazleton Research Animals, Alice, Texas. Upon arrival, the rats and guinea pigs were identifed by metal eartags. The identification numbers (tattooing performed by supplier) on the chest of the monkeys were confirmed and recorded. The animals were housed in environmentally controlled rooms with 10 to 15 air changes per hour. Temperature and humidity were maintained at 72 ± 2°F and 50 ± 10%, respectively, except for brief excursions outside these ranges. The rooms were kept on a 12-h light/dark cycle per day. The rats and guinea pigs were housed in quarantine for at least 7 days prior to use. The monkeys were acclimated for 1 month in quarantine, during which time they were subjected to tuberculin tests 11 days after arrival and 1 month later. All monkeys tested negative for tuberculosis.

During quarantine, the rats and guinea pigs were housed in polycarbonate cages on Ab-Sorb-Dri® (Ab-Sorb-Dri Company, Garfield, New Jersey) hardwood chip bedding. The rats were provided with Purina Certified Rodent Chow (Ralston Purina Company, Richmond, Indiana). The guinea pigs were fed Purina Certified Guinea Pig Chow, and their diets were supplemented with ascorbic acid (0.4 g/L) in the drinking water. The monkeys were housed in stainless steel cages and were fed Purina Certified Primate Chow, No. 5048, supplemented with apples. Tap water was available at all times. No contaminants were known or assumed to have been present in the food or water which could have interfered with or affected the results of this study.

The attendant veterinarian examined the animals during the quarantine period. All animals were determined to be in good health as evidenced by normal growth and appearances, and absence of clinical signs. Prior to testing, the rats and guinea pigs were selected at random using a body weight stratification procedure. The number of rodents used, their ages and body weights are summarized below:

Study	Species	No. of animals	Age[a] (days)	Weight[a] (g)
Washing efficiency (dermal)	Rat	6	79[b]	195-252[b]
	Rat	6	56	169-227
	Guinea pig	6	66	436-588
Preliminary (dermal and i.v.)	Rat	3	56	187-208
	Guinea pig	3	45	357-384
Definitive (dermal and i.v.)	Rat	30	56-72	149-218
	Guinea pig	24	37-46	323-456

[a] Upon initiation of dosing.
[b] Initial study.

Two monkeys were randomly assigned to each treatment group for the first washing efficiency study. The same monkeys were used for the same treatments in the second washing efficiency study. For the definitive study, one monkey from each of the two treatment groups was chosen for treatment either dermally or intravenously. One of these two monkeys were then chosen for the second definitive study. This is summarized below along with ages and body weights:

		Treatment			
Experiment	Date	Soap and water wash	Acetone and water wash	Age (years)	Weight (kg)
Washing efficiency (dermal)	8/27/84[a]	No. 529T No. 604T	No. 554T No. 619T	3.2 to 3.7	4.6 to 5.9
	10/10/84	No. 529T No. 604T	No. 554T No. 619T	3.4 to 3.8	4.9 to 6.4
		Dermal	Intravenous		
Definitive (dermal and i.v.)	11/27/84	No. 604T No. 619T	No. 529T No. 554T	3.5 to 3.9	4.7 to 6.4
	1/16/85	No. 604T	No. 529T	3.8 to 4.1	4.7 to 6.4

[a] Initial studies.

Care and handling of animals was in accordance with the guidelines in "Guide for the Care and Use of Laboratory Animals," 1978 Revision, prepared by the Institute of Laboratory Animal Resources, DHEW Publication No. (NIH) 78-23, and the MRI manual for animal care.

B. Chemicals

Nonlabeled methylenedianiline (Lot No. 1707PK) was purchased from Aldrich Chemical Company, Milwaukee, Wiconsin, and 100 g of the material was received by Midwest Research Institute (MRI) on January 27, 1984. Ring labeled ^{14}C-methylenedianiline (Lot No. 83-25-78) was prepared by the Radiochemical Synthesis Section of MRI. The compound was received June 15, 1984, and September 20, 1984, as the free base (3.2 mCi) in 100% ethanol or as the hydrochloride (8.18 mCi) with a specific activity of 14.8 mCi/mmol. The hydrochloride was converted to the base when required. The radiochemical purity was found to be > 99% as determined by high pressure liquid chromatography and thin-layer chromatography. (For details of synthesis and analysis, see Appendix VI.)

C. Dosage and Treatment

Two dose levels were used in these studies, a "low" dose, ~ 2 mg/kg, and a "high" dose, ~ 20 mg/kg. Appropriate mixtures of the ^{14}C-labeled and nonlabeled 4,4'-MDA were dissolved in an ethanol:water mixture and were applied to the skin or administered intravenously (i.v.) to rats, guinea pigs, and monkeys.

For dermal treatment, the doses were administered in a mixture of ethanol:water (95:5) and applied at a volume of about 0.25 to 0.5 mL/kg (0.1 mL/rat, 0.25 mL/guinea pig, and 1.25 mL/monkey). For monkeys, the volume used in the initial dermal washing efficiency study was 2.5 mL/monkey. The backs of the rats and guinea pigs and the lateral forearm of the monkeys were lightly shaven with electric clippers shortly before dosing. The dose was applied with a disposable micropipette on a specific area (2 cm^2 for rats, 5 cm^2 for guinea pigs, and 50 cm^2 for monkeys) on the freshly shaven skin. Except when indicated otherwise (see experimental design), the dosed areas were covered with a wax-lined cup held in place by adhesive tape.

For intravenous treatment, a mixture of the ^{14}C-labeled and nonlabeled 4,4'-MDA was dissolved in ethanol:water (25:75) and administered at the low dose level in a volume of ~ 2.0 mL/kg (0.4 mL/rat and 1.0 mL/guinea pig) or ~ 1.0 mL/kg (5.0 mL/monkey). Intravenous dosing for all three species was performed through the saphenous vein.

Aliquots of each dosing solution were counted to determine the amounts of radioactivity. These amounts are summarized below:

Study	Species	Dose (~ mg/kg)	Route	Radioactivity (μCi/animal)	Specific activity (dpm/μg)
Washing efficiency (dermal)	Rat	2	Dermal	8.7-10.4	48,511-57,769
	Guinea pig	2	Dermal	26.0	57,769
	Monkey[a]	2	Dermal	115.2	25,568
	Monkey	2	Dermal	127.8	28,374
Preliminary (dermal and i.v.)	Rat	2	Dermal	10.9	60,489
		20	Dermal	11.4	6,347
		2	i.v.	12.3	68,185
	Guinea pig	2	Dermal	31.3	69,491
		20	Dermal	26.6	5,894
		2	i.v.	33.6	74,680
Definitive (dermal and i.v.)	Rat	2	Dermal	25.2	139,299
		20	Dermal	31.2	17,299
		2	i.v.	26.7-32.8	147,971-182,149
	Guinea pig	2	Dermal	43.6	96,873
		20	Dermal	48.0	10,663
		2	i.v.	29.4	65,265
	Monkey	2	Dermal	190.5-231.7	42,281-51,426
		2	i.v.	127.5-259.3	28,308-57,567

[a] Initial study.

D. Experimental Design

1. Washing Efficiency Studies

These studies were performed to assess the efficiency of removal of the applied ^{14}C-labeled material by washing with soap and water or acetone and water. Six rats,* six guinea pigs, and four monkeys** were lightly anesthetized (rats and guinea pigs with an intraperitoneal dose of sodium pentobarbitol, 45 mg/kg and 30 mg/kg, respectively, and monkeys with an intramuscular dose of ketamine hydrochloride, 5 mg/kg). The animals were then treated dermally with 4,4'-MDA at the low dose level. The doses were allowed to dry (5 min) and were then washed with soap and water or acetone and water (three rats, three guinea pigs, and two monkeys for each treatment). Washing was accomplished by soaking a gauze pad in either soap (Sani-Fresh® lotion cleanser, Sani-Fresh International, Inc., San Antonio, Texas) or acetone (certified, Fisher Scientific Company, Fair Lawn, New Jersey) and scrubbing the application areas for ~ 30 s. Gauze pads soaked in tap water were then used to scrub the application areas; this step was repeated five or six times. Each pad was placed in a labeled screw-cap jar and known volumes of methanol were added for extraction of the radioactive material.*** Aliquots (0.25 mL) of these methanol extractions were measured and counted for determination of radioactivity.

Following washing, the application areas were covered and the animals were placed in individual metabolism cages (rats and guinea pigs) or metabolic chairs (for the first 24 h in monkeys) and urine and feces were collected at 6, 12, 24, 48, 72, and 96 h for rats and guinea pigs, and 6, 12, 24, 48, 72, 96, and 168 h for monkeys. At the end of the collection period, rats and guinea pigs were sacrificed by ether inhalation, and the covers and the application areas (skin) were removed and individually extracted with methanol. Later the skins were solubilized in perchloric acid:hydrogen peroxide (2:4) and total radioactivity was determined.

2. Preliminary Studies

These studies were performed to assist in selecting the appropriate sampling times for the definitive studies and to test and assure effectiveness of methods to be used for treatment of rats and guinea pigs. The studies utilized three male rats and three male guinea pigs; one of each species was treated dermally with the low or high dose level of 4,4'-MDA, or i.v. at the low dose level. The application areas were covered (dermally treated), and the animals were placed in individual metabolism cages for collection of urine and feces at 6, 12, 24, 48, 72, and 96 h following dosing. Radioactivity remaining on the skin of dermally treated animals was washed with soap and water as described above and the animals were sacrificed for tissue sampling (see below, Sample Collection).

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* Experiment was repeated due to the low recoveries.

** Experiment was repeated due to the high recovery in the excreta and low recovery in the washing solution.

*** Extraction with methanol was assumed to remove all the radioactivity from the gauze pads.

3. Rat Studies

These studies were performed with 21 rats treated dermally at the low or high dose levels and 9 rats treated i.v. at the low dose level. The dermal dose of 4,4'-MDA was applied and kept on the skin for the duration of the study (6, 24, or 96 h) or kept on the skin for 24 h then removed. After the application area was covered, the animals were placed in metabolism cages for collection of urine and feces at 6, 12, 24, 48, 72, and 96 h. At the specified times, the applied doses were washed with soap and water, then the animals were sacrificed or returned to the metabolic cages for sacrifice at a later time point. A group of three rats were treated dermally at the low dose level, but the application areas were kept nonoccluded by fitting the animal with wax-lined cups with the tops removed; these animals were sacrificed at 6 h.

In the i.v. studies, the rats were treated with the low dose of 4,4'-MDA and were placed in individual metabolism cages for excreta collection at 6, 12, 24, 48, 72, and 96 h following administration. Three animals were sacrificed at 6, 24, or 96 h for tissue sampling.

4. Guinea Pig Studies

These studies were performed with 21 guinea pigs treated dermally at the low or high dose level and 3 guinea pigs treated i.v. The design was similar to the rat studies except that i.v. dosing was limited to one group of three animals which were treated with the low dose and sacrificed at 96 h following dosing.

5. Monkey Studies

In these studies, four male monkeys were treated dermally (2) or i.v. (2) with the low dose of 4,4'-MDA. After dosing, the monkeys were placed in metabolic chairs for the first 24 h. The dermal application areas were then washed with soap and water as described above and the monkeys were transferred to individual metabolism cages for the remainder of the study. Following dosing, urine and feces were collected at 6, 12, 24, 48, 72, 96, 120, 144, and 168 h. One of the dermally treated and one of the i.v. treated monkeys were redosed approximately 6 weeks later to obtain additional excretion data. This study was performed similarly.

E. Sample Collection

Urine was collected in containers kept on dry ice. After each collection, the cages were rinsed and the cage washings were measured and analyzed. At sacrifice, the rodents were anesthetized with ether and exsanguinated by withdrawal of blood from the abdominal aorta. The monkeys were not sacrificed. The following tissues and organs were removed, washed with saline, blotted with absorbing paper, weighed, and prepared for radiochemical analysis:

- Liver
- Kidneys
- Lungs
- Brain
- Spleen
- Adrenals
- Testes
- Urinary bladder
- GI tract plus contents
- Skeletal muscle
- Retroperitoneal fat
- Skin from nontreated areas
- Application area

Portions of blood were centrifuged to separate plasma and red blood cells (RBCs). Bladder contents were removed and the bladder was washed thoroughly with saline. The contents and washings were combined with the final urine samples and analyzed. Blood and tissue were kept on ice during the necropsy procedures. Sample preparation and analyses were performed immediately after collection, or the samples were frozen on dry ice and stored frozen until analyzed. The remaining tissue and excreta were stored frozen.

F. Determination of Total Radioactivity

1. Sample Preparation

Blood, tissue, and excreta were analyzed in duplicate whenever possible. Aliquots (0.1 to 0.25 mL) of the whole blood, RBCs and plasma were analyzed for total radioactivity determination. Volumes of urine and cage rinse were measured, and samples (0.5 to 1.0 mL) were counted. Feces, GI tract (plus contents), and tissues weighing more than 0.25 g were homogenized in 4 volumes of ethanol:water, 10:90. Aliquots of the homogenates were measured and analyzed. Adrenals, bladders, fat, and nontreated skin samples were weighed and assayed for ^{14}C content. The application areas of the skin and the covers were soaked in methanol and the extracted radioactivity was analyzed. Later, the application areas were solubilized with perchloric acid: hydrogen peroxide (2:4) and aliquots were measured and analyzed.* Blood, tissue, and fecal samples were combusted using a Packard Tricarb Oxidizer Model C306. Permafluor V® in combination with Carbo-Sorb® (Packard Instrument Company) was used as the scintillation cocktail. Urine and cage rinse were counted directly in Phase Combining Scintillate (PCS, Amersham).

2. Radioactivity Measurement

Vials were cooled for a minimum of 24 h before counting in a liquid scintillation counter (Packard Tricarb Model 3255). Correction for background was carried out automatically by the counter. Background determinations were obtained from the average of natural counts of several tissue homogenates from nontreated animals. The counting efficiency was determined using the automatic external standard (AES) method. An AES versus efficiency curve was prepared by processing a quench curve set through the counter under the conditions used throughout the experiment. Assays not within ± 10% of the mean of the duplicates were reassayed in duplicate except when the sample was no longer available or when radioactivity counts were low and nonsignificant, i.e., less than two times the background. For these studies, background counts ranged from 25.0 to 35.6 counts/min.

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* Skin solubilization with perchloric acid and hydrogen peroxide was conducted with conditions not likely to cause loss of ^{14}C by oxidation of the ring-^{14}C-labeled 4,4'-MDA to $^{14}CO_2$.

3. Data Processing and Analysis

Carbon-14 contents in blood and tissues are presented in terms of microgram equivalents per milliliter (blood) or gram (tissues) and percentage of the administered dose. The percent of dose excreted in urine and feces is also tabulated. Individual calculations for each sample were performed with an Apple II Plus computer as follows:

1. Cpm (counts per minute) for each sample was converted to dpm (disintegrations per minute).

$$\frac{\text{cpm}}{\text{efficiency}} = \text{dpm/sample}$$

2. Dpm per g or mL was calculated.

$$\frac{\text{dpm/sample}}{\text{sample weight or volume (g or mL)}} = \text{dpm/g or mL}$$

3. Dpm per g or mL was divided by the specific activity of the compound (dpm/μg) to obtain the μg equivalents/g or mL.

$$\frac{\text{dpm/g or mL}}{\text{specific activity}} = \mu\text{g/g or mL}$$

4. This was multiplied by the total weight or volume of the organ or excreta in order to obtain the total amounts in the organ or excreta.

$$\mu\text{g/g or mL} \times \text{total weight or volume} = \mu\text{g/organ or excreta}$$

5. The μg/organ or excreta was divided by the total dose administered in order to obtain the percentage of the administered dose.

$$\frac{\mu\text{g/organ or excreta} \times 100}{\text{total dose (in }\mu\text{g)}} = \text{\% of administered dose}$$

The percent of administered dose recovered in blood, muscle, and skin was calculated based on 7, 40, and 16%, respectively, of body weight. Percent recovery in plasma and RBCs was calculated based on 60 and 40%, respectively, of the total blood volume. These estimates were based on data from the published literature.

IV. RESULTS

A. Dermal Washing Efficiency Studies

Prior to conducting the dermal disposition studies, initial washing efficiency experiments were performed to assess the extent of removal of the applied ^{14}C-labeled material by washing with soap or acetone solutions. Six rats, six guinea pigs, and four monkeys were lightly anesthetized, then treated dermally with low doses (~ 2 mg/kg) of ^{14}C-labeled 4,4'-MDA. After allowing 5 to 10 min for the applied doses to dry, the application areas were washed with soap and water or with acetone and water (three rats, three guinea pigs, and two monkeys for each treatment). After washing, the rats and guinea pigs were placed in individual metabolism cages for excreta collection. Monkeys were kept in metabolism chairs for 24 h before placing in individual metabolism cages. Urine and feces were collected at 6, 12, 24, 48, 72, and 96 h following application. In monkeys, excreta collection was continued for 168 h.

The data generated from the dermal wash studies are summarized in Table 1. Data from individual animals are presented in Appendix II, Table II-1 for rats, II-2 for guinea pigs, and II-3 for monkeys.* In the rat, higher and more consistent recoveries were obtained by washing the application areas with soap and water (91.3%) than with acetone and water (85.1%). In addition, lower amounts were recovered in excreta when soap and water were used: 1.6% after soap and water versus 7.7% after acetone and water. Recoveries of radioactivity from the application areas (methanol extraction, then skin solubilization) were lower after washing with soap and water (3.8%) than after acetone and water (12.7%). Similar results were obtained in guinea pigs; 84.5% of the doses were washable with soap and water while 76.0% were washable with acetone and water. In excreta, 0.6% of the dose was recovered during 96 h following washing with soap and water while 2.1% was recovered after washing with acetone and water. From the application areas, 3.8% was recovered from animals washed with soap and water versus 5.9% after washing with acetone and water.

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* The data shown do not include those generated from the initial rat and monkey studies. The rat studies were repeated to assure that the recoveries obtained in the initial experiments were actual (were similar to those described above for the repeat studies). During 96 h, 1.2% of the dose was recovered in excreta after washing with soap and water versus 6.2% after acetone and water. In the initial studies with monkeys, the animals were placed in metabolism cages following dose application and washing. When the monkey studies were repeated, the animals were kept in monkey chairs for the first 24 h following dose application to minimize the possibility of ingesting any radioactivity remaining on the skin after the application area was washed. In addition, collection of excreta was extended for 168 h to allow for complete elimination before study termination. In this study, slightly higher amounts were recovered in the dose wash by the use of acetone and water, 41.9%, than after washing with soap and water, 36.4%. Similar amounts were recovered in urine and feces, 13.9% after soap and water and 11.5% after acetone and water. The results, however, were more consistent in animals washed with soap and water.

In the monkeys, only 63.0% and 53.0% were recovered in the dose wash after soap and water or acetone and water, respectively. In addition, housing of the monkeys in chairs for 24 h after dose application and washing to prevent any possible ingestion of the radioactivity remaining on the application areas, did not significantly reduce the amounts excreted in urine and feces. In 168 h after washing with soap and water, 8.7% was eliminated in urine and feces. After washing with acetone and water, excretion in urine and feces averaged 16.2%. The total recoveries were still low, 71.7% in monkeys washed with soap and water and 69.2% in monkeys washed with acetone and water. It is possible that significant amounts of the applied doses still remained to be excreted after the 7-day collection period. However, the rate of excretion was slow; approximately 0.5% of the dose was eliminated every 24-h period during the last 3 days of collection. Since no monkey tissues were sampled in this study, it was not possible to determine the location of the unexcreted material.

These studies showed that both soap and water or acetone and water were incapable of removing all the applied material. Significant amounts of the applied doses remained associated with the skin available for absorption. The recoveries were low, especially for monkeys where the study design did not allow for a determination of ^{14}C on the application area or in tissues. In the rat and guinea pig studies, the amounts recovered in the application areas were found to be significant. Even when the dose was washed 24 or 96 h after dermal application (see below), considerable amounts were recovered in the application areas and had to be extracted with methanol followed by skin solubilization.

B. Preliminary Studies

These studies were performed with three rats and three guinea pigs treated dermally with the low or high doses of ^{14}C-labeled 4,4'-MDA or i.v. with the low dose (one animal per treatment). Following dosing, the application areas were covered and the animals were housed in individual metabolism cages for collection of urine and feces at 6, 12, 24, 48, 72, and 96 h. The radioactivity remaining on the skin of dermally treated animals was washed with soap and water (which was selected for dose removal in all preliminary and definitive dermal experiments); then the animals were sacrificed for sampling of selected tissues. As described above, the application areas were removed, processed, and counted. These preliminary studies assisted in selecting the appropriate sampling times for the definitive experiments described below and in determining the methods of treatment and sampling.

The rat data (Tables 2 and 3) showed that most (~ 95%) of the i.v. dose was eliminated in urine and feces during a 96-h period following dosing. After dermal application of the low dose, ~ 43% was eliminated in urine and feces in 96 h. The remainder was recovered in the dose wash, application area, GI tract, and tissues. Although the skin was washed thoroughly, while only 31% was recovered in the dose wash and ~ 24% of the applied dose was recovered from the application area. After dermal application of the high dose, ~ 15% of the dose was recovered in excreta, suggesting limited absorption and/or elimination. About ~ 52% of the high dose was recovered in the dose wash and 32% was removed from the application area.

The guinea pig data (Tables 4 and 5) showed that about 89% of the i.v. dose was recovered in urine and feces in 96 h. After dermal application of the low dose, only 37% of the dose was recovered in excreta following the same period. Slightly lower amounts (∿ 30%) were recovered in excreta following the high dose, but the distribution of ^{14}C in excreta (urine and feces) differed considerably from the animals treated with the low dose. The recoveries in the dose wash and application areas were respectively, ~ 38 and ~ 16% following the low dose, and ~ 49 and ~ 8% following the high dose.

The tissue distribution data obtained from rats and guinea pigs in the preliminary studies are shown in Tables 3 and 5, respectively. Since the data are consistent with those demonstrated in the definitive i.v. and dermal studies which are described below, no discussion of these preliminary data is included.

C. Rat Studies

These studies were performed with nine male Fischer 344 rats treated i.v. with the low dose and 21 rats treated dermally at the low or high doses of ^{14}C-labeled 4,4'-MDA. The rats treated i.v. were housed in individual metabolism cages for excreta collection at 6, 12, 24, 48, 72, and 96 h. At 6, 24, or 96 h, groups of three rats each were sacrificed for tissue sampling.* In the dermal studies, three groups of three rats each were treated with the low doses, the application areas were covered (see methods), and then each rat was placed in a metabolism cage for excreta collection at 6, 12, 24, 48, 72, and 96 h. At 6, 24, or 96 h following treatment, the application areas were washed with soap and water, then the animals were sacrificed for skin and tissue sampling. A fourth group of three rats was treated dermally with the low dose which remained on the skin for 24 h, then removed (washed with soap and water), and the animals returned to the metabolism cages until sacrificed at 96 h. A fifth group of three rats was treated dermally at the low dose and the dosing area was kept uncovered to determine the effect of non-occlusion on the dermal penetration of ^{14}C-labeled 4,4'-MDA. Excreta were collected for 6 h, and then the animals were sacrificed.

For the high dose, two groups of three rats each were treated dermally with the high dose of the test compound. The application areas were covered; then each rat was placed in a metabolism cage for excreta collection at the time periods indicated above. At 24 h, the application areas in one group were washed with soap and water, and the animals were returned to the metabolism cages until rats of both groups were sacrificed at 96 h following dosing.

The data generated from the i.v. studies are summarized in Tables 6 through 9. Data from individual animals are shown in Tables III-1 through III-3 in Appendix III. The data obtained from the dermal studies are shown in Tables 10 through 21. The individual animal data are presented in Appendix III, Tables III-4 through III-14.

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* The studies with rats sacrificed at 6 and 24 h were repeated due to the low recoveries. Data obtained from the initial studies are not included in this report.

1. Radioactivity in Excreta and Tissue

In rats treated i.v., most of the administered doses appeared in urine, the GI tract (probably through bile) and tissues 6 h after dosing. By 24 h, most of the doses were recovered in urine (67.4%) and feces (21.8%). Additional fecal excretion (to 30.7%) occurred between 24 and 96 h (Table 6).

In rats sacrificed at 6 h following continuous dermal application of the low dose, 11.9% of the applied radioactivity was recovered in urine, the GI tract, and tissues. An average of 62.1 and 30.5% were present in the dose wash (soap and water) and the application areas, respectively. For rats sacrificed at 24 h, 27.8% of the dose was recovered in urine, feces, the GI tracts, and tissues; 52.1 and 25.8% were recovered in the dose wash and from the application areas, respectively. By 96 h following continuous dermal exposure to the low dose, 54.7% of the applied ^{14}C was recovered in urine, feces, tissues, and the GI tracts, 24.7% in the dose wash, and 25.6% from the application area.

Rats in which the application areas were kept nonoccluded showed lower amounts of ^{14}C in the excreta, tissues, and the GI tracts (7.0% of the dose in 6 hr) compared to the rats with occluded skin (Table 17). The remainder of the applied doses were recovered in the dose wash (70.2%) or from the application areas (27.5%).

In rats treated dermally with the low dose and the application areas washed at 24 h before the animals were returned to the metabolism cages for an additional 72 h, 42.9% of the doses were recovered in urine, feces, tissues, and the GI tracts. An average of 52.1% of the applied doses were recovered in the dose wash at 24 h. In addition, an average of 10.7% was recovered from the application areas at 96 h, i.e., 72 h after these areas were washed (Tables 14 to 17).

In rats treated dermally with the high dose which was kept on the skin for 96 h, only 6.6% of the applied doses were recovered in urine, feces, GI tracts, and tissues. An average of 62.5% of the doses were washable and 24.0% recovered from the application areas. In the rats treated with the high dose, then the doses washed 24 h later, similar amounts (5.3%) were recovered in excreta and tissues during the 96-h collection period (Tables 18 through 21).

2. Tissue Distribution

Tissue distribution studies were performed in the rats treated i.v. or dermally and sacrificed at 6, 24, or 96 h after dosing. Following an i.v. dose, significant amounts of the radioactive doses (23.9% at 6 h) were recovered in the GI tract. The elimination in the GI tract, probably through bile, contributed to the significant fecal excretion demonstrated at 24 and 96 h (21.8 and 30.7%, respectively). Significant amounts of the radioactive doses (19.4%) were recovered in blood and tissues at 6 h, which declined to 1.5% at 96 h. The highest concentrations were present in the liver, which contained 9.5% of the dose at 6 h and was five times higher than blood (see Table 8).

After continuous dermal application of the low dose, 3.8 and 4.7% were recovered in the GI tract and tissues, respectively, at 6 h. As shown after i.v. dosing, the liver showed the highest concentration. Higher tissue concentrations were demonstrated following the high dose of 4,4'-MDA, although the proportion of dose recovered in tissues was lower than found after the low dose. The distribution in tissues, including liver, was however, similar after the low and high doses. In animals treated dermally with the doses removed 24 h later, a rapid decline in tissue concentrations was demonstrated during the 72-h recovery period, although liver-to-blood ratios remained high (about 7).

3. Recoveries and Dose Balance

The recoveries of radioactivity in blood, tissues, and excreta following treatment of rats with the low dose of ^{14}C-labeled 4,4'-MDA are shown in Tables 9 (i.v. studies), 13, and 17 (dermal studies). Recoveries following dermal application of the high dose are shown in Table 21. The recoveries of the i.v. doses ranged from 98 to 99% of the administered doses. Also, after dermal application of the low doses, quantitative recoveries (104 to 106%) were obtained. Slightly lower recovery (92 to 93%) was obtained following dermal application of the high dose. To achieve these recoveries, the portions of dose remaining on the application areas following washing with soap and water were determined. Initially, the skin was extracted over a 24-h period with methanol, but complete extraction was not achieved. The radioactivity that remained associated with the skin following extraction was determined by skin solubilization using perchloric acid and hydrogen peroxide. Significant amounts of radioactivity were recovered in the skin application area following the different treatments.

The summary data presented for the dermally treated rats include the amounts which were considered absorbed through the skin (Tables 13, 17, and 21). These amounts were obtained by addition of percentages of the doses recovered in the blood, tissue, GI tract, and excreta. Radioactivity associated with the application areas was not included. However, since the ^{14}C recovered from these areas appears to be strongly bound to the skin and is probably available for systemic circulation, it may be considered in calculating the total amounts absorbed. These calculations are presented below.

In rats treated dermally with the low dose, the amounts absorbed (recovered in excreta and tissue) averaged 11.9, 27.8, and 54.5% of the applied doses in animals sacrificed at 6, 24, and 96 h, respectively. When the radioactivity in the dermal application areas was considered in calculating the absorbed amounts, these values increased to 42.5, 53.6, and 80.1% of the applied doses. In rats with the dermal dose kept nonoccluded, the amounts absorbed averaged 7.0 and 34.5% without and with the application areas, respectively. In rats receiving the low dose which was removed 24 h later, the total absorption during a 96-h period averaged 42.9 and 53.0% without and with the application areas, respectively. The rats treated dermally with the high dose absorbed 6.6% of the dose in the continuously treated animals and 5.3% of the dose in animals with the dose washed at 24 h. When the application areas were included, these amounts increased to 30.6 and 14.2%, respectively.

D. Guinea Pig Studies

These studies were performed with three guinea pigs treated intravenously (i.v.) and 21 male guinea pigs treated dermally with ^{14}C-labeled 4,4'-MDA. The design was similar to the rat studies except that i.v. dosing was limited to only three animals which were treated with the low dose of the test compound and sacrificed at 96 h following dosing. The data from the i.v. studies are shown in Tables 22 through 25. Data from individual guinea pigs are presented in Appendix IV, Tables IV-1 through IV-3. The data obtained from the dermal studies are shown in Tables 26 through 37. The individual animal data are presented in Appendix IV, Tables IV-4 through IV-14.

1. Radioactivity in Excreta and Tissue

In guinea pigs treated i.v., most of the administered doses appeared in feces and urine by 48 h following dosing (Table 22) with the highest rate of excretion occurring between 12 and 24 h. By 96 h, recovery in feces (probably through bile) averaged 56.5% and in urine 35.0%.

In guinea pigs sacrificed at 6 hr following continuous application of the low dose, 3.2% of the applied radioactivity was recovered in urine, feces, GI tract, and tissues. An average of 80.6% and 11.4% was present in the dose wash and the application areas, respectively. For guinea pigs sacrificed at 24 h, 17.5% of the dose was recovered in excreta and tissues; 58.8 and 14.8% were recovered from the dose wash and the application areas, respectively. By 96 h following dermal application of the low dose, 39.9% of the dose was recovered in excreta and tissue. As noted following i.v. dosing, fecal elimination represents the primary excretory route in guinea pigs treated dermally with the test compound. An average of 40.9% of the dose was recovered in the dose wash and 29.4% was accounted for in the application areas (Table 29).

Guinea pigs in which the application areas were kept nonoccluded showed similar amounts in excreta, tissues, and the GI tract (3.1%) as animals with the occluded skin (Table 33). The remainder of the applied doses were recovered in the dose wash (87.5%) or from the application areas (9.4%).

In guinea pigs treated dermally with the low dose and the applied doses removed at 24 h before the animals returned to their metabolism cages for an additional 72 h, 18.0% of the doses were recovered in excreta, tissue, and the GI tract. An average of 61.8% was recovered in the dose wash at 24 h and 16.6% was associated with the skin application areas at 96 h (72 h after washing).

In guinea pigs treated dermally with the high dose and sacrificed at 96 h following dosing, only 7.5% of the applied dose was recovered in excreta, tissue, or the GI tracts. An average of 69.9% of the dose was recovered in the dose wash and 13.7% was present in the application areas. Slightly lower amounts (4.2%) were recovered in excreta and tissues of guinea pigs that were treated with the high dose and with the doses washed 24 h later. Recoveries in the dose wash and application areas averaged 80.4 and 7.3%, respectively.

2. Tissue Distribution

Tissue distribution studies were performed in the guinea pigs treated i.v. and sacrificed at 96 h or dermally and sacrificed at 6, 24, or 96 h after dosing. At 96 h after the i.v. dose, only 0.6% of the dose was recovered in the GI tract and 2.9% was recovered from blood and tissue. Elimination in the GI tract at earlier times was probably significant since fecal elimination at 96 h totaled ~ 56% of the dose. In contrast to rats, the guinea pigs showed the highest tissue contents in the spleen (six times higher than blood), which was followed by the liver (four times higher than blood). Blood and liver contents were higher than demonstrated for rats.

After dermal dosing of the guinea pigs, a different tissue distribution pattern was noted at the low and high doses of the test compound. Most notable was the very high concentrations in adrenals (up to 23 times higher than blood) which were demonstrated at 6, 24, and 96 h after dosing. As in the i.v. dosed animals, the concentrations in the liver were also high, but the ^{14}C levels in spleen were considerably lower. The high adrenal levels were also demonstrated in the guinea pigs treated dermally at the high dose with the dose removed at 24 h. In contrast, after discontinuing the exposure to the low dose at 24 h, the levels of ^{14}C in adrenals were significantly reduced (see Tables 28, 32, and 36).

3. Recoveries and Dose Balance

Recoveries of the radioactivity in blood, tissue, and excreta following treatment of guinea pigs with the low dose of ^{14}C-labeled 4,4'-MDA are shown in Tables 25 (i.v. dose), 29, and 33 (dermal doses). The recoveries following dermal treatment with the high dose are shown in Table 37. About 95% of the i.v. dose was recovered in blood, tissue, GI tract, and excreta at 96 h following dosing. After dermal application of the low dose, recoveries ranged from 91 to 100%. Slightly lower recoveries (91 to 92%) were obtained from guinea pigs treated dermally with the high dose. As shown in the rat dermal studies, the recoveries for guinea pig experiments included the dermal application areas which were extracted and solubilized to determine the radioactivity strongly associated with the skin.

The amounts considered to be absorbed through the skin of guinea pigs are included in the recovery tables (Tables 29, 33, and 37). These amounts were obtained from recoveries in the blood, tissue, GI tract, and excreta. As described in the rat studies, ^{14}C in the application areas was not included in the absorption estimates, although the radioactivity recovered from these areas was strongly associated with the skin. In guinea pigs treated dermally with the low dose, 3.2, 17.5, and 29.9% of the doses were absorbed (recovered in excreta and tissue) during 6, 24, and 96 h after dosing. By including the application areas in the absorbed estimates, these amounts increased to 14.6, 32.3, and 59.3%. In guinea pigs with the dermal dose kept nonoccluded, the amounts absorbed averaged 3.1 and 12.6% without and with the application areas, respectively. In animals receiving the low dose which was removed at 24 h, the total absorption during a 96-h period following application averaged 18.0 and 34.6% without and with the application areas, respectively. The guinea pigs treated dermally with the high dose absorbed 7.5% of the dose in the continuously treated animals and 4.2% in the animals with the dose removed at 24 h. When the application areas are included, these amounts increased to 21.2 and 11.5%, respectively.

E. Monkey Studies

In these studies six male rhesus monkeys were treated dermally (three) or i.v. (three) with the low dose of ^{14}C-labeled 4,4'-MDA.* The monkeys, which were acclimated in metabolism chairs prior to dosing, were kept in their chairs during the first 24 h after treatment. At this time, the dermal application areas were washed with soap and water and all monkeys were transferred to individual metabolism cages for the remainder of the study. Urine and feces were collected at 6, 12, 24, 48, 72, 96, 120, 144, and 168 h following dosing. The animals dosed i.v. were treated similarly but skin washing was not performed. In these studies, no tissues were sampled; the monkeys were retained for future investigation following recovery periods.

Table 38 summarizes the elimination data obtained from both groups of monkeys treated i.v. or dermally. The data from individual animals are presented in Appendix V, Table V-1. Almost complete recovery (93 to 98%) was obtained from the monkeys treated i.v. Excretion of ^{14}C occurred primarily in urine (84.3%) and to a lesser extent in feces (9.8%). Peak elimination occurred at 6 to 12 h, although one animal showed considerable ^{14}C elimination between 24 and 48 h. For the dermally treated monkeys, only 36 to 56% (average 47.3%) of the applied doses was recovered in the washing solutions. An average of 18.8% were excreted in urine and 1.4% in feces during the 7-day collection period. Excretion peaked at 24 h for one monkey and at 48 h for the other two monkeys. Total recoveries in skin wash and excreta averaged only 68% (62 to 73%).

Based on the excretory data only, the amounts considered absorbed through monkey skin averaged 20.8% of the dose. Since the application areas were not sampled, it was not possible to determine the total amounts absorbed or available for absorption. On the other hand, ^{14}C associated with the application areas and tissues can be estimated to be 32% of the dose if quantitative recovery (100%) is achievable. If this estimate is considered, the amounts absorbed or available for absorption increase to 52.8% of the applied dose.

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* Since only four monkeys were used in the present studies, two experiments were performed. The first included four monkeys treated dermally or i.v. (two each), and the second included two of the same monkeys treated dermally or i.v. (one each). A recovery period of 50 days after dosing was allowed between the two experiments.

V. DISCUSSION

The possibility of percutaneous penetration of environmental toxicants increases the importance of dermal exposure risk assessment and prevention. The penetration process is more complex than previously thought, and some early generalizations regarding this process are being reassessed. Little research has been published on the relevance of experimental models to man.[2,11–15] In addition, percutaneous absorption of chemicals that come in contact with the skin depends on many parameters, including the concentration of applied dose, the vehicle, and the surface area of application.[24,42,43,46] Variations in absorption also occur due to the anatomical site of application, occlusion, skin condition, and multiple applications. Therefore, the study design of a skin absorption study in any species must take into consideration these and other variables.

With the extensive use of 4,4'-MDA and other aromatic amines, dermal exposure to these compounds seems obvious. Information on the dermal disposition of 4,4'-MDA is not available. Dermal penetration data on other related aromatic amines are also limited.[5,10,19,26,34] The present studies were, therefore, undertaken to assess the disposition characteristics of 4,4'-MDA following its dermal and intravenous administration to three animal models. Our objective in this study was to determine the dermal absorption, systemic elimination, and dermal washing efficiency of 4,4'-MDA. The animal models selected were the rat, a species for which historical data on the toxicity and carcinogenicity of aromatic amines are available and which is used extensively in dermal absorption studies, and the guinea pig and monkey, species which have been shown to have some relevance to humans as models for percutaneous absorption. In these studies, our goals were to establish experimental protocols and data by which the ultimate objective may be reached.

Percutaneous absorption *in vivo* is usually determined by measuring radioactivity in excreta following topical application of a radioactive dose. The amount of radioactivity retained in the body or excreted by some route not assayed (e.g., feces, expired air, sweat) is corrected by determining the amount of radioactivity excreted in urine,[37] following parenteral administration. Blood (or plasma) radioactivity can also be measured and the percent absorption determined by the ratio of the areas under the blood concentration versus time curves following topical and i.v. administration. This method has generally given results similar to those obtained from urinary excretion data.[42] Determination of percutaneous absorption by those methods does not take into account the differences in the route and rates of disposition arising from differences in routes of administration.

Another approach that can be used in determining *in vivo* percutaneous absorption is to measure the loss of the applied radioactive material from the surface as it penetrates the skin.[42] The difference between the application and the residual dose is assumed to be the amount of the chemical absorbed. Interpretation of these data is sometimes difficult since total recovery from the skin is never assured. In addition, the skin may act as a reservoir for unabsorbed material.[22] While this method is not generally utilized to generate dermal penetration data, it obviously offers direct measurements that can reduce underestimations for dermal permeability obtained from indirect measurements of excretion or blood levels. In addition, if the dermal application area is washed thoroughly, the nonrecovered radioactivity probably represents material that either is absorbed or strongly associated with the skin, and is available for later absorption. In fact, when the dermal absorption was measured by sectioning the skin for autoradiographic measurements, the amounts deposited in the skin were considered as absorbed material.[10]

In the present studies, both urinary and fecal elimination were assessed. In addition, in the rat and guinea pig studies, blood, tissues, and skin application areas were assayed. The dermal absorption of 4,4'-MDA was quite extensive, ranging from approximately 21% of the applied doses in the monkey to about 30 and 54% in the guinea pig and rat, respectively. Additionally, when the topical dose in guinea pigs was increased, the dermal absorption of 4,4'-MDA also showed some increases. Therefore, the potential for large quantities of 4,4'-MDA entering the systemic circulation via the dermal route is quite high. These, however, are the lowest estimates for percutaneous absorption of 4,4'-MDA since only amounts excreted or recovered in tissue were considered. When the amounts of radioactivity associated with the skin that could not be removed by washing are included and considered as absorbed or available for systemic absorption, the estimate for percutaneous absorption of 4,4'-MDA is considerably higher.

The data for 4,4'-MDA are similar qualitatively to those developed for other aromatic amines in rats. The low absorption (percent of dose) demonstrated for 4,4'-methylene-bis-ortho-chloro-aniline (MBOCA) could be related to the higher dosage used in the MBOCA studies.[19] On the other hand, the higher absorption demonstrated for benzidine could be due to the lower dosage and larger application areas used in the studies.[34] Our data extend the findings in rats to other species and examine the effects of dosage, concentration, occlusion and washing procedures which were not assessed in detail for MBOCA or benzidine in the published studies.

A comparison between the disposition of 4,4'-MDA administered i.v. to rats, guinea pigs, and monkeys showed that in 96 h, rats eliminated 67% of the dose in urine, and 31% in the feces (ratio of about 2:1). In guinea pigs, the reverse occurred, 35% in urine and 57% in feces. In monkeys, most of the radioactivity (83%) was eliminated in urine with only limited amounts (9%) recovered in feces. Elimination data obtained following dermal application also varied between species. Urinary and fecal elimination in rats averaged 33 and 9%, respectively (about 4:1 ratio). In guinea pigs, the ^{14}C distribution in urine and feces (7 and 11%) was similar to that after i.v. dosing. Also, in monkeys, the distribution of radioactivity in urine and feces (20 and 2%, respectively) was similar to that after i.v. administration.

While the effects of increased dose levels (and concentration) on the absorption, distribution, metabolism, and elimination of xenobiotics have been extensively studied following oral and parenteral administration, only limited studies have been reported following dermal application.[45] The effect of increased dose levels on dermal penetration was examined in the present studies. While both doses utilized (2 and 20 mg/kg) were relatively low, a significant difference in their disposition behavior was demonstrated. In rats, 54 and 7% of the low and high doses, respectively, were absorbed during a 96-h period. If ^{14}C recovered from the application areas is considered, the absorption estimates increase to 80% following the low doses and 31% following the high dose. Similar data were demonstrated for the guinea pig; 30 and 7% were absorbed following the low and high doses, respectively. When radioactivity associated with the application areas is included, these values increase to 59 and 21%, respectively. Although smaller percentages of the high doses were absorbed, the absolute amounts (in µg/animal) were similar (rats) or slightly higher (guinea pigs) than after the low doses. Since in the published literature many of the dermal penetration experiments were conducted with high doses of the test compounds, which are sometimes higher than encountered under human exposure conditions, the data from these studies may significantly underestimate the amounts absorbed. In some studies the doses utilized (on a mg/kg basis) were not specified and, therefore, it is difficult to interpret the findings of these studies. Dose-dependent absorption and/or elimination may not, however, apply to the disposition of all chemicals.

Immediate washing upon skin contamination is recommended for most industrial chemicals since it is assumed that washing will remove the chemical from the exposed area. In the present study, extensive washing procedures immediately after 4,4'-MDA application failed to remove all the applied doses. A significant portion of these doses remained available for delayed absorption. A post-exposure wash at 6 h, 24 h, or 96 h also failed to remove all the 4,4'-MDA that was not absorbed. Presumably the remaining ^{14}C had been strongly associated with or was "irreversibly" bound to the skin. These findings and those recently reported for polychlorinated biphenyls[36] indicate that postcontamination washing cannot be assumed to remove all chemical from the skin. In addition, dermal washing may not only fail to remove skin contaminated chemical, but it can also promote percutaneous absorption of the chemical.[42] This was demonstrated in our study by the increased absorption of 4,4'-MDA following washing with acetone and water.

In the present studies, some differences were noted in the skin penetration of 4,4'-MDA in the three animal models examined. Depending on how dermal absorption is estimated, these differences can be significant. In all cases, however, the rat showed higher absorption than the guinea pig and monkey, which showed comparable absorption. The relation of these data to human absorption of 4,4'-MDA is not known and cannot be accurately assessed. Data related to the dermal absorption of aromatic amines in animal species are limited, and in humans are almost absent. At present, the animal models of choice for assessing dermal penetration of aromatic amines are not known. The animal model of choice may vary depending on the compound of interest. A comparison to human skin should be made using *in vitro* methods which compare the *in vitro* permeability of skin from humans and animals and relate the findings to the data obtained *in vivo* from these animal models.

VI. CONCLUSIONS AND RECOMMENDATIONS

1. Under the conditions of the present study, dermal absorption of ^{14}C-labeled 4,4'-MDA was extensive in the three animal models used: the rats, the guinea pigs, and the monkeys. Under similar conditions of exposure, comparable absorption was demonstrated in the guinea pig and the monkey, and higher absorpton in the the rat.

2. The studies in rats and guinea pigs, which were performed at two dose levels (concentrations), showed only limited or no differences in the absolute amounts absorbed (in µg/animal) by increasing the dose 10 times. Although the percent of absorbed doses was significantly reduced by increasing the applied dose, the rate of absorption (in µg/h) remained the same in rats and was doubled in guinea pigs.

3. The present studies clearly indicate that washing of the skin following 4,4'-MDA application did not remove all of the applied material. Some remained associated with the skin and available for later absorption and systemic circulation. The available data indicate that 4,4'-MDA or its metabolites are released from the skin and recovered in excreta. Neither washing with soap or acetone solutions which are generally used in industrial settings were capable of removing all the applied material. In addition, washing with acetone facilitated 4,4'-MDA absorption in the three species examined. It is possible that other related organic solvents behave in a way similar to acetone.

4. Distribution of radioactivity in excretory products differed in the three species examined. In monkeys, most of the dose was excreted in urine. In rats, two-thirds of the dose was eliminated in urine, while in guinea pigs two-thirds of the radioactivity was excreted in feces. The proportions of ^{14}C eliminated in urine and feces after dermal application were similar to i.v. dosing in monkeys and guinea pigs. Rats, however, showed a higher proportion of the dose in urine. Similarly, while tissue concentrations in rats were similar after i.v. and dermal dosing, with the highest levels shown in the liver, significant differences were noted in guinea pigs. After i.v. dosing the highest tissue levels in guinea pigs were demonstrated in the spleen while after dermal application, the highest levels were present in the adrenals.

5. Some *in vivo* methods used for calculating dermal absorption should be used with caution. Calculating dermal absorption by relating the urinary excretion following dermal and parentral dosing assumes that the compound or metabolites will be eliminated in a similar way after both routes of administration. While this appears to be the case for some compounds or in some species, e.g., 4,4'-MDA in guinea pigs, this is not always accurate. Relating blood levels only after dermal and i.v. dosing can also generate significantly higher absorption values at early times. It is possible that data obtained by calculating area under blood (plasma) concentration values would offer a more accurate procedure, but this was not examined in the present study.

6. These data suggest considerable absorption of 4,4'-MDA in humans exposed dermally to 4,4'-MDA. The rate and extent of this absorption, however,

cannot be accurately assessed from the present studies. Since it is not possible to test 4,4'-MDA absorption directly in humans, the available data developed with animal models _in vivo_ along with data using animal and human excised skin _in vitro_ would provide a good estimate on the rate and extent of human skin penetration of 4,4'-MDA.

7. Also, the available _in vivo_ information on 4,4'-MDA compared with _in vitro_ data on 4,4'-MDA and other related aromatic amines would assist in establishing a definitive structure activity relationship between these compounds. The _in vitro_ studies can also assist in assessing the effect of different conditions of application including dose, concentration, vehicle, etc., on the penetration processes.

8. While the present studies provided skin penetration data for 4,4'-MDA in several animal species and assessed the effect of washing on removal of this compound from the skin, it provided no means for qualitative analysis of the circulating or excretory metabolic product of 4,4'-MDA. Knowledge of such products is essential for direct assessment of workers or consumer exposure to 4,4'-MDA. Most aromatic amines are metabolized and only limited amounts are excreted unchanged. Qualitative data on the excretory products in different species along with studies in exposed workers would assist in establishing animal models most relevant to man.

9. No data are available on the disposition (absorption, ditribution, and elimination) or metabolism of 4,4'-MDA administered orally (by gavage or feed). Such information is essential to the interpretation of data on the toxicity and carcinogenicity of this compound which was demonstrated following oral administration to rodents.

VII. SUMMARY

A. Objective and Study Design

Studies were performed with the overall objective of developing dermal penetration data for 4,4-methylenedianiline (4,4'-MDA), an aromatic amine with known toxic and carcinogenic properties. Techniques for measuring dermal absorption rates were selected and compared. The studies were performed in vivo using ^{14}C-labeled 4,4'-MDA in three animal models: the Fischer rat, Hartley guinea pig, and Rhesus monkey. Several conditions of exposure including dosage (low and high), exposure regimens (limited or continuous), and occlusion status (exposed or covered) were addressed. In addition, studies were conducted to determine the dermal washing efficiency of 4,4'-MDA following its application in these species. Limited experiments were also performed to assess the disposition of the test compound following intravenous (i.v.) administration. The dermal absorption studies in rats and guinea pigs were performed at two dose levels, "low" and "high," while the i.v. studies were performed at the low dose. The monkey studies were carried out with the low dose only. All application areas were kept occluded except in selected studies. The low dose used was ~ 2 mg/kg (0.4 mg/rat, 1.0 mg/guinea pig, and 10.0 mg/monkey). The high dose was ~ 20 mg/kg (4.0 mg/rat and 10.0 mg/guinea pig). The test compound concentration was 4 mg/mL for the low dose and 40 mg/mL for the high dose. The application areas were 2 cm^2 for rats (~ 200 g each) 5 cm^2 for guinea pigs (~ 500 g each) and 50 cm^2 for monkeys (~ 5000 g each). Ethanol (95%) was used for the dermal studies while ethanol:water (25:75) was utilized as the vehicle for the i.v. studies.

B. Dermal Washing Efficiency Studies

These experiments were performed to assess the extent of removal of dermally applied ^{14}C-labeled 4,4'-MDA by washing with soap and water or with acetone and water. Six male rats, six male guinea pigs, and four male monkeys were treated dermally with low doses of the test compound, then the application areas were washed 5 to 10 min later with either solution. After washing, the animals were housed in individual metabolism cages for excreta (urine and feces) collection during a 4-day (rats and guinea pigs) or 7-day period (monkeys). At termination the skin application areas (from rats and guinea pigs only) were removed, extracted with methanol, then solubilized. Radioactivity was measured in the washing solutions, in excreta, and in the application areas.

The studies in the three species showed that higher and more consistent recoveries were obtained by washing the application areas with soap and water versus acetone and water (Table A). Recoveries (percent of applied dose) in the dose wash were 91.3% versus 85.1% (of applied dose) in rats, 84.5% versus 76.0% in guinea pigs, and 63.0% versus 53.0% in monkeys. In addition, lower amounts were recovered in excreta after washing with soap and water than with acetone and water: 1.6 versus 7.7% in rats, 0.6 versus 2.1% in guinea pigs, and 8.7 versus 16.2% in monkeys. The studies showed that both washing methods were incapable of removing all the applied material from the skin; significant amounts of the applied doses remained on the skin available for absorption. In addition, the results suggested that acetone facilitated absorption during the washing process. In rats and guinea pigs, the skin application areas contained higher amounts of radioactivity after washing with acetone and water than after washing with soap and water (17.7 versus 3.8% in rats and 5.9 versus 3.8% in guinea pigs). The total recoveries were lower for monkeys since the application areas were not sampled. Removal of the applied doses from monkey skin appeared more difficult than from rat or guinea pig skin as evidenced by the lower recoveries in the washing solutions and the higher recoveries in excreta.

C. Rat Studies

These studies were performed with male rats treated dermally at the low or high doses of ^{14}C-labeled 4,4'-MDA or i.v. at the low dose. After dermal application, the rats were placed in metabolism cages for excreta collection until sacrificed at 6, 24, or 96 h for tissue sampling. At sacrifice, the application areas were washed with soap and water. Two additional groups were treated dermally with the low or high doses and the applied doses were washed 24 h later, and then excreta collection continued until the animals were sacrificed at 96 h. Another group was treated dermally with the low dose and the application area was kept nonoccluded until sacrifice 6 h later.

In rats treated dermally with the low doses which were kept on the skin for the duration of the study, 11.9, 27.8, and 54.5% of the applied doses were recovered in excreta and tissue at 6, 24, and 96 h after dosing; 62.1, 52.1, and 24.7% were washed from the application areas and 30.6, 25.8, and 25.6% were recovered from the skin following methanol extraction and skin solubilization (Table B). In rats treated dermally with the low dose for 24 h then the dose removed, recoveries in tissue and excreta at 96 h averaged 42.9%. In the dose wash, 52.1% was recovered at 24 h and 10.7% remained associated with the skin 72 h after washing (Table C). In rats with the application areas nonoccluded, 7.0% of the low dose was recovered in the excreta and tissue at 6 h, 70.2% was recovered in the dose wash, and 27.5% was associated with the skin application area.

In rats treated dermally with the high doses which were kept on the skin for 96 h, only 7.5% of the applied doses were recovered in tissue and excreta during this period. Slightly lower amounts (5.3%) were recovered during the same period when the dose was washed 24 h after application. In the first group (continuous application), 62.5% of the doses were removed with soap and water at 96 h and 24.0% recovered from the application area. In the second group (limited application), 77.9% was removed at 24 h and 8.9% recovered from the application area at 96 h (Tables B and C).

In the i.v. studies, most of the administered doses were recovered in excreta (urine and feces) by 24 h after dosing. Total recoveries in excreta and tissue averaged 98.3, 98.1, and 99.3% at 6, 24, and 96 h after dosing (Table B). During 96 h following i.v. dosing, 67.0 and 30.7% of doses were eliminated in urine and feces, respectively. During the same time period, rats treated dermally with the low dose for 96 h excreted 43.0 and 10.0% of doses in urine and feces, respectively. Following the high doses, excretion in urine and feces during a 96-h period averaged 4.8 and 1.3%, respectively.

Tissue distribution studies following i.v. dosing showed that significant amounts of the radioactivity (~ 24% at 6 h) were recovered in the GI tract, probably through the bile. Blood and tissue contained ~ 19% of the doses at 6 h, but their ^{14}C contents declined to ~ 2% of the doses at 96 h. The highest ^{14}C concentrations were demonstrated in the liver, which was five to nine times higher than blood. After continuous dermal application of the low dose, ~ 4% was recovered in the GI tract and similar amounts were present in blood and tissues. Also, the liver showed the highest concentration (four to nine times higher than blood). Distribution in tissue was similar after the high dose, but the proportion of doses (percent of doses) recovered in tissues was lower than after the low dose.

D. Guinea Pig Studies

These studies were performed with male guinea pigs treated dermally with the low or high doses of ^{14}C-labeled 4,4'-MDA or i.v. at the low dose. The study design was similar to the rat studies except that i.v. dosing was limited to one group of guinea pigs sacrificed at 96 h. After dosing, the animals were placed in metabolism cages for excreta collection until sacrificed at 6, 24, or 96 h for tissue sampling. At sacrifice, the application areas were washed with soap and water. As in the rat studies, two additional groups were treated dermally with the low or high doses and the application areas were washed 24 h later; then the animals were sacrificed at 96 h. In addition, a group of guinea pigs were treated dermally with the low dose and the application areas were kept nonoccluded until the animals were sacrificed 6 h later.

In guinea pigs treated dermally with the low doses which were kept on the skin for the duration of the study, 3.2, 17.5, and 29.9% of the doses were recovered in excreta and tissue at 6, 24, and 96 h after dosing. At these times, 80.6, 58.8, and 40.9% were recovered in the dose wash and 11.4, 14.8, and 29.4% remained associated with the application area (Table D). In guinea pigs treated dermally with the low dose and dose removed at 24 h, recoveries in tissue and excreta at 96 h averaged 18.0%; 61.8% was present in the dose wash at 24 h and 16.6% was recovered from the skin at 96 h. In animals with the application areas nonoccluded, 3.1% of the dose was recovered in the excreta and tissue, 87.5% was recovered in the dose wash, and 9.4% was associated with the skin application area.

After dermal application of the high dose which was kept on the skin for 96 h, only 7.5% of the applied doses were recovered in excreta and tissue during this period. Slightly lower amounts (4.2%) were recovered during the same period when the doses were washed 24 h after application. In the first group (continuous application), 69.7% of the dose was washable with soap and water at 96 h and 13.7% recovered from the application area. In the second group (limited application), 80.4% of the dose was washed at 24 h and 7.3% was recovered from the application area at 96 h (Table E).

Following i.v. dosing of guinea pigs with ^{14}C-labeled 4,4'-MDA, most of the dose was recovered in urine and feces by 48 h after dosing. Recoveries in excreta and tissue averaged 95.0% at 96 h after 4,4'-MDA administration (Table D). During 96 h following i.v. dosing, 35.0 and 56.5% of doses were eliminated in urine and feces, respectively. Guinea pigs treated dermally with the low dose for 96 h excreted 10.5 and 17.6% of the doses in urine and feces, respectively, during the same period. Excretion in urine and feces during a 96 h period averaged 2.8 and 3.6% after dermal application of the high dose.

Tissue distribution studies showed that only limited amounts were recovered in the GI tract (0.6%), blood, and tissue (2.9%) at 96 h after i.v. dosing. With the high fecal elimination after i.v. dosing, excretion into the GI tract at earlier times appeared significant. The highest ^{14}C contents were demonstrated in the spleen (six times higher than blood) followed by the liver. Blood and liver contents were higher in guinea pigs than in rats. After dermal dosing, a different tissue distribution pattern was noted. Very high concentrations were noted in the adrenals (up to 23 times higher than blood) at all sacrifice times following dermal application of the low or high doses. The concentrations in liver were also higher than blood, but ^{14}C levels in the spleen were low. When exposure to the test compound was discontinued by washing the dose at 24 h, animals treated with the high dose maintained high adrenal contents, while these contents were significantly reduced after discontinuing (washing off) the low dose.

E. Monkey Studies

These studies were performed with male monkeys treated dermally or i.v. with the low dose of ^{14}C-labeled 4,4'-MDA. Following dosing, the monkeys were kept in metabolism chairs for 24 h, the skin was washed, and then each monkey was housed in a metabolism cage for excreta collection during a 168-h period. The monkeys were not sacrificed and, therefore, the skin applications areas were not sampled. In the dermally treated monkeys, 20.8% of the applied doses were eliminated in the urine and feces during the 168-h period. Recovery during a 96 h period averaged 18.0%. Only 47.3% of the dose was recovered in the dose wash at 24 h after treatment. The total recoveries for these animals averaged 68% of the dose, which indicated that significant portions of the doses remained on the skin available for later absorption. In contrast, recoveries were almost quantitative in the monkey treated i.v.; an average of 94.1% of the dose was excreted during the 7-day collection period. Most of the radioactivity was excreted by 48 h following dosing (see Table G). Following both i.v. and dermal administration, ^{14}C excretion in monkeys occurred primarily in urine. Urinary and fecal excretion averaged 84.4 and 9.8%, respectively, after i.v. dosing, and 18.8 and 1.9%, respectively, after dermal application.

F. Conclusions

Under the conditions of these studies, dermal penetration of 4,4'-MDA was extensive in the three animal models examined. The absorbed radioactivity was generally distributed throughout the body and was readily eliminated in urine and feces. Association of 4,4'-MDA and/or its metabolites to the skin was also significant, resulting in low washing efficiency with soap or acetone solutions. After washing, considerable amounts of the applied radioactivity remained associated with the skin available for later absorption. When only the amounts of radioactivity excreted in urine and feces or recovered in blood and tissue were considered in determining dermal absorption, the rats demonstrated the highest absorption followed by the guinea pigs and the monkeys.

When the amounts of radioactivity associated with the skin were considered in calculating 4,4'-MDA dermal absorption, comparable absorption was demonstrated for the rats, guinea pigs, and monkeys.

The distribution of radioactivity recovered in urine and feces differed in the three species, allowing only direct comparison of data obtained by both routes of elimination. Although in monkeys the application areas were washed at 24 h following treatment and the collection period was extended to 7 days, direct comparison between the three species was possible since the study design incorporated groups of rats and guinea pigs in which the dose was removed at 24 h following application. Under these comparable conditions of treatment, 52, 62, and 47% of the applied doses were washable at 24 h in rats, guinea pigs, and monkeys, respectively. Total amounts excreted in urine and feces during 96-h periods were 42, 17, and 18% in these three species. These data indicate comparable absorption in monkeys and guinea pigs and higher absorption in rats. As expected, maximum absorption (based on percent of applied dose) occurred in animals following continuous treatment with the low dose and with the application areas remained occluded. These maximum amounts averaged 54% in rats and 30% in guinea pigs during a 96-h period. In monkeys, the maximum absorption (determined by urinary and fecal elimination only) averaged 21% of the applied doses in 168 h. The total amounts excreted in urine and feces and recovered in tissues provided more accurate estimates of dermal absorption than the amounts obtained by measuring urinary excretion and correcting for recovery from the parenterally administered doses. Assessment of absorption based on recovery of ^{14}C in blood and/or tissue only did not provide accurate measurements of the amounts absorbed.

While increasing the 4,4'-MDA applied doses (and concentration) resulted in lower percentages of the doses that penetrated the skin, the absolute amounts absorbed (in µg/animal) remained the same (in rats) or increased (in guinea pigs) by increasing the dose. In rats, increasing the applied dose 10 times resulted in a decrease of percent of dose absorbed from 54.5 to 6.6%, but the average absorption rate over a 96 h period was similar (2.3 µg/h and 2.8 µg/h after the low and high doses, respectively). In guinea pigs, absorption decreased from 29.9% after the low dose to 7.9% after the high dose, but the absorption rate increased from 3.1 to 8.0 µg/h (Table H). Occulsion increased 4,4'-MDA dermal absorption in rats (11.9 versus 7.0%) but had no effect on its dermal penetration in guinea pigs (3.2 versus 3.4%). However, the studies performed to assess occlusion were limited to 6-h sampling periods and, therefore, definitive conclusions cannot be reached. When 4,4'-MDA applied to the skin was washed immediately with soap and water or acetone and water, as low as 53% was recovered in the washing solutions. A post-exposure wash at 6 h, 24 h, and 96 h removed less of the applied material. Post-contamination washing cannot be assumed to remove all 4,4'-MDA from the skin.

Table A. Dermal Washing Efficiency of 4,4'-MDA in Rats, Guinea Pigs, and Monkeys[a,b]

	Percent of dose					
	Soap and water wash			Acetone and water wash		
	Rats	G. Pigs	Monkeys	Rats	G. Pigs	Monkeys
Urine[c]	1.21	0.33	7.25	5.89	1.36	14.47
Feces[c]	0.35	0.31	1.49	1.80	0.76	1.76
Dose wash	91.33	84.52	62.98	85.09	76.04	53.00
Application area[d]	3.80	3.83	ND	12.71	5.91	ND
Recovery	96.68	88.99	71.71	105.49	84.07	69.23

[a] Applied doses were ~2 mg/kg (0.4 mg/rat, 1.0 mg/guinea pig, and 10.0 mg/monkey). The doses were washed 5-10 min after application.

[b] Mean of three for rats and guinea pigs and 2 for monkeys.

[c] Excreta collected for 96 h in rats and guinea pigs and 168 h in monkeys.

[d] Application areas of skin were removed, processed, and counted. Monkey skin was not sampled.

Table B. Recovery of Radioactivity in Rats Treated i.v or Dermally with 4,4'-MDA[a]

	Percent of dose						
	0.4 mg/rat						4 mg/rat
	i.v.			Dermal[b]			Dermal[b]
	6 h	24 h	96 h	6 h	24 h	96 h	96 h
Blood	2.80	0.44	0.22	0.77	0.29	0.11	0.03
Tissue	16.60	4.33	1.31	4.70	2.15	0.88	0.22
GI tract	23.94	4.14	0.11	3.80	2.98	0.48	0.18
Urine	54.65	67.35	66.96	2.55	20.03	43.04	4.82
Feces	0.28	21.79	30.66	0.04	2.31	9.96	1.34
Total absorbed[c]	98.27	98.06	99.25	11.85	27.76	54.47	6.58
Dose wash[d]	-	-	-	62.08	52.08	24.67	62.45
Application area[e]	-	-	-	30.59	25.84	25.55	24.00
Recovery	98.27	98.06	99.25	104.51	105.68	104.68	93.02

[a] Mean of three rats per group.

[b] Continuous application for the period indicated.

[c] Recovered in blood, tissue, GI tract, and excreta.

[d] Removed by washing with soap and water.

[e] Removed by methanol extraction and skin solubilization.

Table C. Recovery of Radioactivity in Rats Treated Dermally with 4,4'-MDA under Different Conditions[a]

	Percent of dose		
	0.4 mg/rat		4.0 mg/rat
	6 h/ nonoccluded	24 h wash/ 96 h sacrifice	24 h wash/ 96 h sacrifice
Blood	0.53	0.07	0.01
Tissue	3.26	0.38	0.13
GI tract	1.24	0.08	0.07
Urine	1.95	33.23	4.00
Feces	0.03	9.18	1.06
Total absorbed[b]	7.00	42.93	5.27
Dose wash[c]	70.21	52.12	77.92
Application area[d]	27.53	10.67	8.93
Recovery	104.74	105.72	92.11

[a] Mean of 3 rats per group.
[b] Recovered in blood, tissue, and excreta.
[c] Removed by washing with soap and water.
[d] Removed by methanol extraction and skin solubilization.

Table D. Recovery of Radioactivity in Guinea Pigs Treated i.v. or Dermally with 4,4'-MDA[a]

	Percent of dose				
	1.0 mg/G. pig				
	96 h/ i.v.	6 h/ dermal[b]	24 h/ dermal[b]	96 h/ dermal[b]	10 mg/G. pig 96 h/dermal[b]
Blood	0.55	0.12	0.14	0.14	0.04
Tissue	2.39	1.11	1.02	1.08	0.43
GI tract	0.61	1.51	2.80	0.59	0.64
Urine	34.98	0.35	7.81	10.47	2.75
Feces	56.45	0.10	5.70	17.62	3.62
Total absorbed[c]	94.98	3.19	17.46	29.89	7.48
Dose wash[d]	-	80.55	58.78	40.85	69.70
Application area[e]	-	11.43	14.77	29.41	13.72
Recovery	94.98	95.16	91.01	100.14	90.89

[a] Mean of three guinea pigs per group.
[b] Continuous application of the period indicated.
[c] Recoved in blood, tissue, and excreta.
[d] Removed by washing with soap and water.
[e] Removed by methanol extraction and skin solubilization.

Table E. Recovery of Radioactivity in Guinea Pigs Treated Dermally with 4,4'-MDA under Different Conditions[a]

	Percent of dose		
	1.0 mg/G. pig		10 mg/G. pig
	6 h/ nonoccluded	24 h wash/ 96 h sacrifice	24 h wash/ 96 h sacrifice
Blood	0.12	0.08	0.02
Tissue	1.02	0.33	0.13
GI tract	1.27	0.12	0.28
Urine	0.60	6.56	1.54
Feces	0.12	10.89	2.18
Total absorbed[b]	3.14	17.98	4.15
Dose wash[c]	87.46	61.78	80.44
Application area[d]	9.37	16.58	7.29
Recovery	99.97	96.34	91.88

[a] Mean of three guinea pigs per group.
[b] Recovered in blood, tissue, and excreta.
[c] Removed by washing with soap and water.
[d] Removed by methanol extraction and skin solubilization.

Table F. Recovery of Radioactivity in Monkeys Treated i.v. or Dermally with 4,4'-MDA[a]

	Percent of dose	
	10.0 mg/monkey	
	168 h/i.v.	168 h/dermal[b]
Urine	84.34	18.83
Feces	9.75	1.93
Total absorbed[c]	94.09	20.76
Dose wash[d]	-	47.27
Recovery	94.09	68.03

[a] Mean of three monkeys, per group.
[b] The dose was washed at 24 h after application.
[c] Recovered in excreta. Tissues and application areas of skin were not sampled.
[d] Removed by washing with soap and water.

Table G. Percutaneous Absorption of 4,4'-MDA Based on Urinary Excretion Data Only

Time (h)	Cumulative % of dose in urine		% Absorption Dermal/i.v. x 100	Absorption rate[a]	
	I.V.	Dermal		% dose/h	µg/h
Rats, Low Dose (0.4 mg/Rat)					
6	36.24	1.51	4.2	0.70	2.8
12	55.10	9.77	17.7	1.48	5.9
24	63.84	23.45	36.7	1.53	6.1
48	65.61	36.11	55.0	1.14	4.6
72	66.51	40.69	61.2	0.85	3.4
96	66.96	43.04	64.3 (54.5)[b]	0.67	2.7
Rats, High Dose (4 mg/Rat)					
6	36.24[c]	0.15	0.4	0.07	2.8
12	55.10	0.52	0.9	0.08	3.2
24	63.84	1.19	1.9	0.08	3.2
48	65.61	2.58	3.9	0.08	3.2
72	66.51	3.77	5.7	0.08	3.2
96	66.96	4.82	7.2 (6.6)[b]	0.08	3.2
Guinea Pigs, Low Dose (1.0 g/Guinea Pig)					
6	6.43	0.22	3.4	0.57	5.7
12	21.76	1.52	7.0	0.58	5.8
24	30.65	4.32	14.1	0.59	5.9
48	33.96	7.64	22.5	0.47	4.7
72	34.64	9.13	26.4	0.37	3.7
96	34.98	10.47	29.9 (29.9)[b]	0.31	3.1
Guinea Pigs, High Dose (10 mg/Guinea Pig)					
6	6.43[c]	0.04	0.6	0.10	10.0
12	21.76	0.30	1.4	0.12	12.0
24	30.65	0.54	1.8	0.08	8.0
48	33.96	0.94	2.8	0.06	6.0
72	34.64	1.63	4.7	0.06	6.0
96	34.98	2.75	7.9[c] (7.5)[b]	0.05	5.0
Monkey Low Dose (10 mg/Monkey)					
6	24.85	0.19	0.8	0.13	13
12	45.95	1.01	2.2	0.18	18
24	63.69	3.84	6.0	0.25	25
48	79.35	11.20	14.1	0.29	29
72	82.48	15.12	18.3	0.25	25
96	83.27	16.75	20.1 (18.1)[b]	0.21	21
120	83.64	17.66	21.1	0.18	18
144	83.90	18.30	21.8	0.15	15
168	84.34	18.83	22.3 (20.8)[b]	0.13	13

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[a] Average rate calculated from cumulative absorption based on the urinary excretion data.

[b] Recoveries in urine, feces, and tissue (where applicable).

[c] I.V. treatment with the low dose only.

Table H. Percutaneous Absorption of 4,4'-MDA Based on Recoveries in Excreta, Tissue, and Skin Application Areas

Group	% of dose in Excreta and Tissue	Absorption rate		Skin Application Area[a]	Total
		% dose/hr	µg/h		
Rats, Low Dose (0.4 mg/Rat)					
6 h	11.85	1.98	7.92	30.59	42.44
24 h	27.76	1.16	4.64	25.84	53.60
96 h	54.47	0.57	2.28	25.55	80.02
24/96 h	42.93	0.45	1.80	10.67	53.59
6 h nonoccluded	7.00	1.17	4.68	27.53	34.53
Rats, High Dose (4 mg/Rat)					
96 h	6.58	0.07	2.80	24.00	30.58
24/96 h	5.27	0.05	2.00	8.93	14.20
Guinea Pigs, Low Dose (1.0 mg/Guinea Pig)					
6 h	3.19	0.53	5.3	11.43	14.62
24 h	17.46	0.73	7.3	14.77	32.23
96 h	29.89	0.31	3.1	29.41	59.30
24/96 h	17.98	0.19	1.9	16.58	34.56
6 h nonoccluded	3.14	0.52	5.2	9.37	12.51
Guinea Pigs, High Dose (10 mg/Guinea Pig)					
96 h	7.48	0.08	8.0	13.72	21.20
24/96 h	4.15	0.04	4.0	7.29	11.44
Monkeys, Low Dose (10 mg/Monkey)					
24/96 h	18.04[b]	0.18	18.0	-	-
24/168 h	20.76[b]	0.12	12.0	(32.00)[c]	52.76

[a] Remaining after skin washing with soap and water.
[b] Excreta only.
[c] Skin application area and tissue as estimated from differences in recovery.

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Table 1. Dermal Washing Efficiency of ^{14}C-Labeled 4,4'-MDA in Male Fischer 344 Rats, Hartley Guinea Pigs, and Rhesus Monkeys[a]

		Percent of dose					
		Soap and water wash			Acetone and water wash		
Excretum	Time after dosing (h)	Rats[b]	Guinea pigs[b]	Monkeys[c]	Rats[b]	Guinea pigs[b]	Monkeys[c]
Urine	6	0.23 ± 0.09	0.04 ± 0.02	0.28	0.65 ± 0.07	0.14 ± 0.07	0.33
	12	0.41 ± 0.11	0.06 ± 0.02	0.42	2.38 ± 0.91	0.22 ± 0.15	0.69
	24	0.24 ± 0.04	0.10 ± 0.03	1.39	1.38 ± 0.53	0.34 ± 0.11	1.73
	48	0.15 ± 0.01	0.07 ± 0.01	1.65	0.75 ± 0.11	0.37 ± 0.05	5.36
	72	0.08 ± 0.02	0.04 ± 0.01	1.49	0.37 ± 0.11	0.19 ± 0.06	2.81
	96	0.09 ± 0.03	0.02 ± 0.01	0.69	0.36 ± 0.09	0.11 ± 0.02	1.54
	120			0.53			0.81
	144			0.40			0.76
	168			0.41			0.46
Feces	6	0.01 ± 0.00	0.00 ± 0.00	0.00	0.01 ± 0.00	0.00[c]	0.00
	12	0.05 ± 0.03	0.01 ± 0.00	0.01	0.09 ± 0.04	0.08 ± 0.07	0.00
	24	0.06 ± 0.01	0.13 ± 0.05	0.02	0.32 ± 0.14	0.21 ± 0.04	0.06
	48	0.14 ± 0.02	0.10 ± 0.03	0.26	0.57 ± 0.06	0.19 ± 0.09	0.43
	72	0.05 ± 0.00	0.04 ± 0.01	0.36	0.37 ± 0.15	0.19 ± 0.04	0.46
	96	0.04 ± 0.01	0.03 ± 0.00	0.28	0.45 ± 0.19	0.10 ± 0.01	0.30
	120			0.22			0.22
	144			0.16			0.12
	168			0.19			0.16
		Cumulative percent of dose					
Urine	6	0.23 ± 0.09	0.04 ± 0.02	0.28	0.65 ± 0.07	0.14 ± 0.07	0.33
	12	0.64 ± 0.20	0.10 ± 0.03	0.70	3.03 ± 0.88	0.36 ± 0.22	1.02
	24	0.89 ± 0.23	0.20 ± 0.06	2.08	4.41 ± 1.41	0.69 ± 0.31	2.75
	48	1.03 ± 0.24	0.28 ± 0.07	3.74	5.15 ± 1.51	1.06 ± 0.35	8.11
	72	1.11 ± 0.25	0.31 ± 0.07	5.23	5.52 ± 1.61	1.26 ± 0.39	10.91
	96	1.21 ± 0.28	0.33 ± 0.08	5.91	5.89 ± 1.68	1.36 ± 0.41	12.45
	120			6.44			13.26
	144			6.84			14.01
	168			7.25			14.47
Feces	6	0.01 ± 0.00	0.00 ± 0.00	0.00	0.01 ± 0.00	0.00[c]	0.00
	12	0.06 ± 0.03	0.01 ± 0.01	0.01	0.10 ± 0.04	0.08 ± 0.07	0.00
	24	0.12 ± 0.04	0.14 ± 0.05	0.03	0.42 ± 0.15	0.29 ± 0.10	0.07
	48	0.26 ± 0.05	0.23 ± 0.08	0.28	0.99 ± 0.20	0.48 ± 0.16	0.49
	72	0.31 ± 0.05	0.28 ± 0.08	0.64	1.36 ± 0.35	0.66 ± 0.19	0.95
	96	0.35 ± 0.05	0.31 ± 0.08	0.92	1.80 ± 0.52	0.76 1 0.20	1.25
	120			1.14			1.47
	144			1.30			1.59
	168			1.49			1.76
Dose wash		91.33 ± 2.95	84.52 ± 3.27	62.98	85.09 ± 6.17	76.04 ± 4.13	53.00
Application area		3.80 ± 0.16	3.83 ± 0.57	ND[d]	12.71 ± 1.14	5.91 ± 0.65	ND[d]
Recovery		96.68 ± 2.90	89.00 ± 2.94	71.71	105.49 ± 4.27	84.07 ± 3.06	69.23

[a] The compound was applied at a dose of 0.4 mg/rat, 1.0 mg/guinea pig, and 10.0 mg/monkey.

[b] Mean ± SE of three animals per group.

[c] Average of two animals.

[d] Not determined.

Table 2. Urinary and Fecal Excretion of Radioactivity in Male Fischer 344 Rats Treated Dermally or Intravenously with ^{14}C-Labeled 4,4'-MDA (0.4 or 4.0 mg/Rat): Preliminary Study

		Percent of dose			Cumulative percent of dose		
Excretum	Time after dosing (h)	0.4 mg dermal No. 13	4.0 mg dermal No. 14	0.4 mg i.v. No. 15	0.4 mg dermal No. 13	4.0 mg dermal No. 14	0.4 mg i.v. No. 15
Urine	6	0.54	0.14	15.77	0.54	0.14	15.77
	12	7.47	0.62	30.09	8.01	0.76	45.86
	24	8.88	1.78	13.35	16.89	2.54	59.21
	48	11.03	2.39	2.02	27.93	4.93	61.23
	72	4.42	3.46	0.66	32.34	8.39	61.89
	96	2.64	3.49	0.56	34.98	11.88	62.45
Feces	6	0.00	0.00	0.11	0.00	0.00	0.11
	12	0.06	0.00	0.36	0.07	0.01	0.47
	24	0.68	0.08	14.91	0.75	0.08	15.37
	48	3.78	1.09	16.37	4.53	1.17	31.74
	72	1.76	1.11	0.65	6.29	2.28	32.39
	96	1.18	1.30	0.38	7.47	3.58	32.78
Total	6	0.55	0.14	15.88	0.55	0.14	15.88
	12	7.53	0.62	30.45	8.08	0.76	46.33
	24	9.57	1.86	28.26	17.64	2.62	74.59
	48	14.82	3.48	18.39	32.46	6.10	92.97
	72	6.18	4.57	1.31	38.64	10.67	94.28
	96	3.82	4.79	0.94	42.45	15.46	95.23

Table 3. Radioactivity in Blood, Tissue, and Excreta of Male Fischer 344 Rats at 96 h Following a Dermal or Intravenous Dose of ^{14}C-Labeled 4,4'-MDA (0.4 or 4.0 mg/Rat): Preliminary Study

Tissue/ excretum	μg equivalents/g or mL			Percent of dose		
	0.4 mg dermal No. 13	4.0 mg dermal No. 14	0.4 mg i.v. No. 15	0.4 mg dermal No. 13	4.0 mg dermal No. 14	0.4 mg i.v. No. 15
Blood[a]	0.03	0.20	0.07	0.09	0.07	0.26
Plasma[a,b]	0.02	0.21	0.04	0.05	0.04	0.10
RBCs[a,b]	0.03	0.13	0.09	0.04	0.09	0.13
Liver	0.17	0.73	0.35	0.47	0.18	0.97
Kidneys	0.03	0.24	0.05	0.02	0.01	0.04
Lungs	0.01	0.13	0.17	0.00	0.00	0.05
Brain	0.00	0.04	0.00	0.00	0.00	0.00
Spleen	0.01	0.08	0.16	0.00	0.00	0.03
Testes	0.01	0.08	0.01	0.00	0.01	0.00
Adrenals	0.04	0.33	0.10	0.00	0.00	0.00
Bladder	0.00	0.05	0.00	0.00	0.00	0.00
Muscle[a]	0.00	0.04	0.00	0.05	0.08	0.07
Fat[b]	0.01	0.09	0.01	-	-	-
GI tract	0.01	1.13	0.02	0.42	0.75	0.15
Nontreated skin[a]	0.02	0.40	0.01	0.15	0.30	0.09
Urine	-	-	-	34.98	11.88	62.45
Feces	-	-	-	7.47	3.58	32.78
Dose wash	-	-	-	31.36	51.72	-
Application area	-	-	-	24.17	31.56	-
Recovery	-	-	-	99.20	100.13	96.89

[a] Percent of dose calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[b] Individual blood components and fat are not included in recovery estimates.

Table 4. Urinary and Fecal Excretion of Radioactivity in Male Hartley Guinea Pigs Treated Dermally or Intravenously with ^{14}C-Labeled 4,4'-MDA (1 or 10 mg/Guinea Pig): Preliminary Study

Excretum	Time after dosing (h)	Percent of dose 1 mg dermal No. 52	Percent of dose 10 mg dermal No. 53	Percent of dose 1 mg i.v. No. 54	Cumulative percent of dose 1 mg dermal No. 52	Cumulative percent of dose 10 mg dermal No. 53	Cumulative percent of dose 1 mg i.v. No. 54
Urine	6	0.41	0.08	2.80	0.41	0.08	2.80
	12	1.54	3.05	18.43	1.95	3.13	21.24
	24	5.63	14.11	6.23	7.58	17.24	27.46
	48	8.32	3.97	3.56	15.90	21.21	31.02
	72	2.87	1.58	0.74	18.77	22.79	31.76
	96	1.93	1.08	0.37	20.70	23.87	32.13
Feces	6	0.10	0.01	0.64	[illegible].10	0.01	0.64
	12	0.53	0.14	20.91	0.62	0.14	21.55
	24	3.19	2.28	18.13	3.81	2.42	39.67
	48	4.05	1.83	11.06	7.86	4.26	50.74
	72	4.05	1.04	4.02	11.91	5.30	54.75
	96	3.93	1.30	1.90	15.83	6.60	56.65
Total	6	0.51	0.09	3.44	0.51	0.09	3.44
	12	2.07	3.19	39.34	2.58	3.27	42.78
	24	8.82	16.39	24.35	11.39	19.66	67.14
	48	12.37	5.80	14.62	23.76	25.47	81.75
	72	6.92	2.62	4.75	30.67	28.09	86.51
	96	5.86	2.38	2.27	36.53	30.47	88.78

Table 5. Radioactivity in Blood, Tissue, and Excreta of Male Hartley Guinea Pigs at 96 h Following a Dermal or Intravenous Dose of ^{14}C-Labeled 4,4'-MDA (1 or 10 mg/Guinea Pig): Preliminary Study

Tissue/ excretum	μg equivalents/g or mL			Percent of dose		
	1 mg dermal No. 52	10 mg dermal No. 53	1 mg i.v. No. 54	1 mg dermal No. 52	10 mg dermal No. 53	1 mg i.v. No. 54
Blood[a]	0.05	0.18	0.18	0.12	0.05	0.46
Plasma[a,b]	0.05	0.18	0.17	0.08	0.03	0.26
RBCs[a,b]	0.05	0.15	0.22	0.05	0.02	0.22
Liver	0.22	0.61	1.22	0.37	0.10	1.89
Kidneys	0.12	0.32	0.28	0.06	0.01	0.12
Lungs	0.03	0.11	0.35	0.01	0.00	0.07
Brain	0.00	0.03	0.00	0.00	0.00	0.00
Spleen	0.02	0.06	2.29	0.00	0.00	0.10
Testes	0.06	0.08	0.03	0.01	0.00	0.00
Adrenals	0.64	1.79	0.21	0.01	0.00	0.00
Bladder	0.01	0.02	0.01	0.00	0.00	0.00
Muscle[a]	0.01	0.05	0.01	0.12	0.07	0.08
Fat[b]	0.02	0.13	0.03	-	-	-
GI tract	0.22	0.94	0.11	1.10	0.50	0.83
Nontreated skin[a]	0.04	0.68	0.06	0.24	0.39	0.35
Urine	-	-	-	20.70	23.87	32.13
Feces	-	-	-	15.83	6.60	56.65
Dose wash	-	-	-	38.39	49.37	-
Application area	-	-	-	15.61	8.31	-
Recovery	-	-	-	92.57	89.27	92.68

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[a] Percent of dose calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[b] Individual blood components and fat are not included in recovery estimates.

Table 6. Urinary and Fecal Excretion of Radioactivity in Male Fischer 344 Rats Treated Intravenously with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)[a]

Excretum	Time after dosing (h)	Percent of dose	
		24-h sacrifice	96-h sacrifice
Urine	6	37.91 ± 4.65	36.24 ± 1.03
	12	20.67 ± 2.34	18.85 ± 1.24
	24	8.78 ± 1.04	8.74 ± 0.62
	48		1.77 ± 0.10
	72		0.90 ± 0.15
	96		0.45 ± 0.01
Feces	6	0.20 ± 0.03	3.11 ± 1.33
	12	4.61 ± 2.81	13.31 ± 0.47
	24	16.99 ± 2.47	11.23 ± 2.07
	48		2.02 ± 0.08
	72		0.51 ± 0.10
	96		0.50 ± 0.17
Total	6	38.10 ± 4.68	39.35 ± 1.95
	12	25.27 ± 2.22	32.16 ± 1.27
	24	25.76 ± 3.23	19.96 ± 2.51
	48		3.79 ± 0.14
	72		1.41 ± 0.24
	96		0.95 ± 0.17
		Cumulative percent of dose	
Urine	6	37.91 ± 4.65	36.24 ± 1.03
	12	58.57 ± 2.70	55.10 ± 0.59
	24	67.35 ± 1.71	63.84 ± 0.07
	48		65.61 ± 0.13
	72		66.51 ± 0.22
	96		66.96 ± 0.22
Feces	6	0.20 ± 0.03	3.11 ± 1.33
	12	4.80 ± 2.84	16.41 ± 0.86
	24	21.79 ± 1.52	27.64 ± 1.25
	48		29.66 ± 1.20
	72		30.17 ± 1.14
	96		30.66 ± 1.05
Total	6	38.10 ± 4.68	39.35 ± 1.95
	12	63.37 ± 5.53	71.51 ± 1.41
	24	89.14 ± 2.73	91.47 ± 1.22
	48		95.26 ± 1.08
	72		96.67 ± 1.03
	96		97.62 ± 0.96

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[a] Mean ± SE of three rats per group.

Table 7. Radioactivity in Blood, Tissue, and Excreta at 6, 24, and 96 h Following Intravenous Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)[a]

Tissue/excretum	µg equivalents/g or mL 6 h	 24 h	 96 h
Blood	0.97 ± 0.06	0.15 ± 0.01	0.06 ± 0.00
Plasma	1.14 ± 0.12	0.16 ± 0.01	0.05 ± 0.00
RBCs	0.47 ± 0.04	0.13 ± 0.01	0.06 ± 0.00
Liver	4.86 ± 0.21	1.38 ± 0.21	0.31 ± 0.02
Kidneys	0.96 ± 0.06	0.09 ± 0.01	0.05 ± 0.01
Lungs	1.15 ± 0.13	0.68 ± 0.24	0.07 ± 0.00
Brain	0.14 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Spleen	0.89 ± 0.06	0.41 ± 0.02	0.20 ± 0.01
Testes	0.63 ± 0.11	0.02 ± 0.00	0.01 ± 0.00
Adrenals	0.80 ± 0.12	0.15 ± 0.03	0.10 ± 0.01
Bladder	0.05 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
Muscle	0.17 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Fat	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
GI tract	5.66 ± 0.28	0.64 ± 0.03	0.02 ± 0.00
Skin	0.48 ± 0.02	0.04 ± 0.01	0.02 ± 0.00
	Percent of administered dose		
Blood	2.80 ± 0.10	0.44 ± 0.03	0.22 ± 0.01
Plasma[b,c]	1.97 ± 0.15	0.28 ± 0.02	0.10 ± 0.01
RBCs[b,c]	0.55 ± 0.04	0.15 ± 0.01	0.09 ± 0.00
Liver	9.47 ± 0.62	3.54 ± 0.50	0.86 ± 0.02
Kidneys	0.48 ± 0.03	0.05 ± 0.00	0.03 ± 0.00
Lungs	0.26 ± 0.04	0.24 ± 0.07	0.02 ± 0.00
Brain	0.08 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Spleen	0.09 ± 0.00	0.06 ± 0.00	0.02 ± 0.00
Testes	0.26 ± 0.03	0.01 ± 0.00	0.00 ± 0.00
Adrenals	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Bladder	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Muscle[b]	2.77 ± 0.25	0.19 ± 0.03	0.21 ± 0.02
GI tract	23.94 ± 1.70	4.14 ± 0.27	0.11 ± 0.02
Skin[b]	3.19 ± 0.18	0.25 ± 0.04	0.16 ± 0.01
Urine	54.65 ± 2.92	67.35 ± 1.71	66.96 ± 0.22
Feces	0.28 ± 0.25	21.79 ± 1.52	30.66 ± 1.05
Recovery	98.27 ± 4.04	98.06 ± 3.04	99.25 ± 1.00

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[a] Mean ± SE of three rats per group.

[b] Calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[c] Individual blood components and fat are not included in recovery estimates.

Table 8. Tissue-to-Blood Concentration Ratios from Male Fischer 344 Rats at 6, 24, or 96 h Following Intravenous Treatment with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)[a]

Tissue	6 h	24 h	96 h
Plasma	1.18	1.07	0.75
RBCs	0.49	0.82	1.03
Liver	5.02	9.05	5.05
Kidneys	0.99	0.61	0.85
Lungs	1.19	4.42	1.16
Brain	0.15	0.05	0.07
Spleen	0.91	2.70	3.34
Testes	0.65	0.10	0.08
Adrenals	0.83	0.96	1.69
Bladder	0.06	0.03	0.07
Muscle	0.17	0.08	0.18
Fat	0.02	0.06	0.10
GI tract	5.85	4.16	0.25
Skin	0.50	0.25	0.33

[a] Derived from mean of three rats per group.

Table 9. Recovery of Radioactivity in Blood, Tissue, and Excreta at 6, 24, or 96 h Following Intravenous Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)[a]

	Percent of dose		
Tissue/excretum	6 h	24 h	96 h
Blood	2.80 ± 0.10	0.44 ± 0.03	0.22 ± 0.01
Tissue	16.60 ± 1.00	4.33 ± 0.47	1.31 ± 0.03
GI tract	23.94 ± 1.70	4.14 ± 0.27	0.11 ± 0.02
Urine	54.65 ± 2.92	67.35 ± 1.71	66.96 ± 0.22
Feces	0.28 ± 0.25	21.79 ± 1.52	30.66 ± 1.05
Recovery	98.27 ± 4.04	98.06 ± 3.04	99.25 ± 1.00

[a] Mean ± SE of three rats per group.

Table 10. Urinary and Fecal Excretion of Radioactivity in Male Fischer 344 Rats Treated Dermally with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)[a]

Excretum	Time after dosing (h)	Percent of dose	
		24-h sacrifice	96-h sacrifice
Urine	6	2.21 ± 0.40	1.51 ± 0.78
	12	4.81 ± 1.64	8.25 ± 1.59
	24	13.02 ± 1.91	13.68 ± 0.30
	48		12.65 ± 1.57
	72		4.58 ± 0.74
	96		2.36 ± 0.36
Feces	6	0.03 ± 0.01	0.02[b]
	12	0.61[b]	0.76 ± 0.19
	24	1.87 ± 0.11	2.69 ± 0.40
	48		3.84 ± 0.55
	72		1.63 ± 0.32
	96		1.03 ± 0.13
Total	6	2.24 ± 0.40	1.52 ± 0.77
	12	5.21 ± 1.86	9.01 ± 1.70
	24	14.89 ± 1.80	16.37 ± 0.53
	48		16.50 ± 2.12
	72		6.21 ± 0.42
	96		3.38 ± 0.49
		Cumulative percent of dose	
Urine	6	2.21 ± 0.40	1.51 ± 0.78
	12	7.01 ± 2.00	9.77 ± 0.82
	24	20.03 ± 3.68	23.45 ± 0.99
	48		36.11 ± 2.43
	72		40.69 ± 3.11
	96		43.04 ± 3.20
Feces	6	0.03 ± 0.01	0.02[b]
	12	0.44 ± 0.23	0.77 ± 0.19
	24	2.31 ± 0.21	3.46 ± 0.45
	48		7.30 ± 0.39
	72		8.93 ± 0.12
	96		9.96 ± 0.25
Total	6	2.24 ± 0.40	1.52 ± 0.77
	12	7.45 ± 2.22	10.54 ± 0.93
	24	22.34 ± 3.80	26.91 ± 0.97
	48		43.41 ± 2.71
	72		49.62 ± 3.12
	96		53.00 ± 3.25

[a] Mean ± SE of three rats per group.
[b] Average of two rats.

Table 11. Radioactivity in Blood, Tissue, and Excreta at 6, 24, and 96 h Following Dermal Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)[a]

Tissue/excretum	μg equivalents/g or mL 6 h	 24 h	 96 h
Blood	0.21 ± 0.04	0.08 ± 0.02	0.03 ± 0.00
Plasma	0.25 ± 0.05	0.09 ± 0.02	0.03 ± 0.00
RBCs	0.12 ± 0.02	0.04 ± 0.01	0.03 ± 0.00
Liver	0.86 ± 0.13	0.55 ± 0.05	0.27 ± 0.01
Kidneys	0.26 ± 0.03	0.12 ± 0.02	0.06 ± 0.01
Lungs	0.13 ± 0.04	0.04 ± 0.01	0.02 ± 0.00
Brain	0.06 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
Spleen	0.10 ± 0.02	0.03 ± 0.01	0.01 ± 0.00
Testes	0.13 ± 0.02	0.04 ± 0.01	0.01 ± 0.00
Adrenals	0.26 ± 0.05	0.10 ± 0.02	0.05 ± 0.01
Bladder	0.01 ± 0.01	0.00[d]	0.01 ± 0.01
Muscle	0.06 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
Fat	0.01 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
GI tract	0.86 ± 0.36	0.74 ± 0.21	0.09 ± 0.01
Nontreated skin	0.15 ± 0.03	0.05 ± 0.01	0.02 ± 0.00
	Percent of administered dose		
Blood[b]	0.77 ± 0.14	0.29 ± 0.06	0.11 ± 0.01
Plasma[b,c]	0.54 ± 0.10	0.18 ± 0.04	0.05 ± 0.01
RBCs[b,c]	0.17 ± 0.03	0.06 ± 0.01	0.04 ± 0.00
Liver	1.96 ± 0.31	1.20 ± 0.12	0.54 ± 0.03
Kidneys	0.15 ± 0.01	0.07 ± 0.01	0.03 ± 0.00
Lungs	0.03 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
Brain	0.03 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
Spleen	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Testes	0.07 ± 0.01	0.02 ± 0.01	0.00 ± 0.00
Adrenals	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Bladder	0.00 ± 0.00	0.00[d]	0.00 ± 0.00
Muscle[b]	1.19 ± 0.18	0.43 ± 0.09	0.10 ± 0.01
GI tract	3.80 ± 1.57	2.98 ± 0.97	0.48 ± 0.07
Nontreated skin[b]	1.24 ± 0.20	0.41 ± 0.10	0.20 ± 0.02
Urine	2.55 ± 0.16	20.03 ± 3.68	43.04 ± 3.20
Feces	0.04 ± 0.01	2.31 ± 0.21	9.96 ± 0.25
Dose wash	62.08 ± 6.90	52.08 ± 6.09	24.67 ± 2.85
Application area	30.59 ± 2.20	25.84 ± 4.00	25.55 ± 0.65
Recovery	104.51 ± 3.60	105.68 ± 3.30	104.68 ± 1.47

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[a] Mean ± SE of three rats per group.

[b] Percent of dose calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[c] Individual blood components and fat are not included in recovery estimates.

[d] Average of two rats.

Table 12. Tissue-to-Blood Concentration Ratios from Male Fischer 344 Rats at 6, 24, or 96 h Following Dermal Treatment with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)[a]

Tissue	6 h	24 h	96 h
Plasma	1.17	1.06	0.83
RBCs	0.56	0.54	0.87
Liver	4.00	6.88	9.10
Kidneys	1.21	1.49	1.97
Lungs	0.60	0.50	0.50
Brain	0.28	0.16	0.10
Spleen	0.47	0.39	0.43
Testes	0.60	0.51	0.23
Adrenals	1.20	1.19	1.70
Bladder	0.07	0.04	0.40
Muscle	0.27	0.26	0.17
Fat	0.07	0.16	0.07
GI tract	4.00	9.23	3.03
Nontreated skin	0.71	0.63	0.80

[a] Derived from mean of three rats per group.

Table 13. Recovery of Radioactivity in Blood, Tissue, and Excreta at 6, 24, or 96 h Following Dermal Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)[a]

	Percent of dose		
Tissue/excretum	6 h	24 h	96 h
Blood	0.77 ± 0.14	0.29 ± 0.06	0.11 ± 0.01
Tissue	4.70 ± 0.74	2.15 ± 0.33	0.88 ± 0.04
GI tract	3.80 ± 1.57	2.98 ± 0.97	0.48 ± 0.07
Urine	2.55 ± 0.16	20.03 ± 3.68	43.04 ± 3.20
Feces	0.04 ± 0.01	2.31 ± 0.21	9.96 ± 0.25
Total absorbed	11.85 ± 2.43	27.76 ± 5.15	54.47 ± 3.27
Dose wash	62.08 ± 6.90	52.08 ± 6.09	24.67 ± 2.85
Application area	30.59 ± 2.20	25.84 ± 4.00	25.55 ± 0.65
Recovery	104.51 ± 3.60	105.68 ± 3.30	104.68 ± 1.47

[a] Mean ± SE of three rats per group.

Table 14. Urinary and Fecal Excretion of Radioactivity Following Dermal Application of ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat) to Male Fischer 344 Rats: Application Area Washed at 24 h[a]

Excretum	Time after dosing (h)	Percent of dose	Cumulative percent of dose
Urine	6	2.35 ± 0.20	2.35 ± 0.20
	12	7.30 ± 0.42	9.65 ± 0.23
	24	15.89 ± 1.17	25.54 ± 1.40
	48	6.23 ± 0.59	31.77 ± 1.99
	72	0.74 ± 0.10	32.51 ± 2.04
	96	0.72 ± 0.17	33.23 ± 1.95
Feces	6	0.03[b]	0.03[b]
	12	0.10 ± 0.04	0.12 ± 0.05
	24	4.45 ± 0.19	4.57 ± 0.14
	48	3.72 ± 0.87	8.29 ± 0.90
	72	0.60 ± 0.09	8.89 ± 0.99
	96	0.29 ± 0.04	9.18 ± 0.98
Total	6	2.37 ± 0.21	2.37 ± 0.21
	12	7.40 ± 0.43	9.77 ± 0.25
	24	20.33 ± 1.21	30.11 ± 1.45
	48	9.95 ± 1.46	40.06 ± 2.89
	72	1.34 ± 0.18	41.40 ± 3.02
	96	1.01 ± 0.20	42.41 ± 2.92

[a] Mean ± SE of three rats per group.
[b] Average of two rats.

Table 15. Radioactivity in Blood, Tissue, and Excreta of Male Fischer 344 Rats Treated Dermally with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat): Nonoccluded Skin and Skin Washed at 24 h[a]

Tissue/excretum	µg equivalents/g or mL Nonoccluded/6-h sacrifice	 24-h wash/96-h sacrifice
Blood	0.15 ± 0.03	0.02 ± 0.00
Plasma	0.15 ± 0.02	0.01 ± 0.00
RBCs	0.11 ± 0.04	0.02 ± 0.00
Liver	0.62 ± 0.06	0.13 ± 0.02
Kidneys	0.17 ± 0.02	0.03 ± 0.00
Lungs	0.09 ± 0.03	0.01 ± 0.00
Brain	0.04 ± 0.00	0.00 ± 0.00
Spleen	0.06 ± 0.00	0.01 ± 0.00
Testes	0.09 ± 0.01	0.00 ± 0.00
Adrenals	0.15 ± 0.02	0.01 ± 0.01
Bladder	0.01 ± 0.01	0.00 ± 0.00
Muscle	0.05 ± 0.00	0.00 ± 0.00
Fat	0.01 ± 0.00	0.00 ± 0.00
GI tract	0.29 ± 0.04	0.03 ± 0.01
Nontreated skin	0.09 ± 0.01	0.01 ± 0.00
	Percent of administered dose	
Blood[b]	0.53 ± 0.09	0.07 ± 0.01
Plasma[b,c]	0.33 ± 0.04	0.03 ± 0.00
RBCs[b,c]	0.16 ± 0.06	0.03 ± 0.00
Liver	1.42 ± 0.09	0.21 ± 0.01
Kidneys	0.10 ± 0.01	0.01 ± 0.00
Lungs	0.02 ± 0.01	0.00 ± 0.00
Brain	0.02 ± 0.00	0.00 ± 0.00
Spleen	0.01 ± 0.00	0.00 ± 0.00
Testes	0.05 ± 0.01	0.00 ± 0.00
Adrenals	0.00 ± 0.00	0.00 ± 0.00
Bladder	0.00 ± 0.00	0.00 ± 0.00
Muscle[b]	0.94 ± 0.02	0.05 ± 0.01
GI tract	1.24 ± 0.18	0.08 ± 0.02
Nontreated skin[b]	0.71 ± 0.09	0.10 ± 0.01
Urine	1.95 ± 0.31	33.23 ± 1.95
Feces	0.03 ± 0.00	9.18 ± 0.98
Dose wash	70.21 ± 7.98	52.12 ± 3.10
Application area	27.53 ± 6.18	10.67 ± 1.44
Recovery	104.74 ± 2.64	105.72 ± 1.28

[a] Mean ± SE of three rats per group.

[b] Percent of dose calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[c] Individual blood components and fat are not included in recovery estimates.

Table 16. Tissue-to-Blood Concentration Ratios from Male Fischer 344 Rats Following Dermal Treatment with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat): Nonoccluded Skin and Skin Washed at 24 h[a]

Tissue	Nonoccluded/6-h sacrifice	24-h wash/96-h sacrifice
Plasma	1.04	0.63
RBCs	0.78	1.21
Liver	4.22	7.00
Kidneys	1.18	1.68
Lungs	0.62	0.37
Brain	0.24	0.05
Spleen	0.39	0.37
Testes	0.59	0.11
Adrenals	1.02	0.47
Bladder	0.05	0.05
Muscle	0.31	0.16
Fat	0.06	0.05
GI tract	1.95	1.47
Nontreated skin	0.59	0.68

[a] Derived from mean of three rats per group.

Table 17. Recovery of Radioactivity in Blood, Tissue, and Excreta Following Dermal Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat): Nonoccluded Skin and Skin Washed at 24 h[a]

	Percent of dose	
Tissue/excretum	Nonoccluded/6-h sacrifice	24-h wash/96-h sacrifice
Blood	0.53 ± 0.09	0.07 ± 0.01
Tissue	3.26 ± 0.21	0.38 ± 0.02
GI tract	1.24 ± 0.18	0.08 ± 0.02
Urine	1.95 ± 0.31	33.23 ± 1.95
Feces	0.03 ± 0.00	9.18 ± 0.98
Total absorbed	7.00 ± 0.77	42.93 ± 2.95
Dose wash	70.21 ± 7.98	52.12 ± 3.10
Application area	27.53 ± 6.18	10.67 ± 1.44
Recovery	104.74 ± 2.64	105.72 ± 1.28

[a] Mean ± SE of three rats per group.

Table 18. Urinary and Fecal Excretion of Radioactivity in Male Fischer 344 Rats Treated Dermally with ^{14}C-Labeled 4,4'-MDA (4.0 mg/Rat): Continuous Application for 96 h versus Skin Washing at 24 h[a]

Excretum	Time after dosing (h)	Percent of dose	
		Continuous	24-h Wash
Urine	6	0.15 ± 0.02	0.08 ± 0.01
	12	0.37 ± 0.05	0.26 ± 0.09
	24	0.67 ± 0.05	0.61 ± 0.05
	48	1.39 ± 0.11	1.45 ± 0.24
	72	1.19 ± 0.21	1.01 ± 0.36
	96	1.05 ± 0.17	0.60 ± 0.21
Feces	6	0.01 ± 0.00	0.00 ± 0.00
	12	0.06 ± 0.01	0.05 ± 0.01
	24	0.17 ± 0.01	0.14 ± 0.03
	48	0.36 ± 0.05	0.37 ± 0.04
	72	0.33 ± 0.06	0.29 ± 0.07
	96	0.41 ± 0.08	0.21 ± 0.07
Total	6	0.16 ± 0.02	0.08 ± 0.00
	12	0.43 ± 0.05	0.30 ± 0.11
	24	0.84 ± 0.05	0.75 ± 0.07
	48	1.75 ± 0.15	1.82 ± 0.26
	72	1.52 ± 0.26	1.30 ± 0.43
	96	1.46 ± 0.25	0.82 ± 0.29
		Cumulative percent of dose	
Urine	6	0.15 ± 0.02	0.08 ± 0.01
	12	0.52 ± 0.05	0.33 ± 0.09
	24	1.19 ± 0.06	0.94 ± 0.12
	48	2.58 ± 0.05	2.39 ± 0.17
	72	3.77 ± 0.25	3.40 ± 0.52
	96	4.82 ± 0.42	4.00 ± 0.73
Feces	6	0.01 ± 0.00	0.00 ± 0.00
	12	0.06 ± 0.01	0.05 ± 0.02
	24	0.24 ± 0.02	0.19 ± 0.04
	48	0.59 ± 0.04	0.56 ± 0.06
	72	0.92 ± 0.10	0.85 ± 0.11
	96	1.34 ± 0.14	1.06 ± 0.19
Total	6	0.16 ± 0.02	0.08 ± 0.00
	12	0.58 ± 0.06	0.38 ± 0.11
	24	1.43 ± 0.07	1.13 ± 0.15
	48	3.17 ± 0.08	2.95 ± 0.20
	72	4.69 ± 0.34	4.25 ± 0.63
	96	6.15 ± 0.56	5.06 ± 0.91

[a] Mean ± SE of three rats per group.

Table 19. Radioactivity in Blood, Tissue, and Excreta of Male Fischer 344 Rats 96 h Following Dermal Treatment with ^{14}C-Labeled 4,4'-MDA (4.0 mg/Rat): Continuous Application for 96 h versus Washing at 24 h[a]

	µg equivalents/g or mL	
Tissue/excretum	Continuous	24-h wash
Blood	0.08 ± 0.02	0.04 ± 0.01
Plasma	0.10 ± 0.02	0.04 ± 0.02
RBCs	0.05 ± 0.01	0.02 ± 0.01
Liver	0.53 ± 0.08	0.35 ± 0.09
Kidneys	0.19 ± 0.06	0.08 ± 0.03
Lungs	0.05 ± 0.01	0.02 ± 0.01
Brain	0.01 ± 0.01	0.00 ± 0.00
Spleen	0.03 ± 0.01	0.01 ± 0.01
Testes	0.04 ± 0.01	0.02 ± 0.01
Adrenals	0.15 ± 0.04	0.05 ± 0.01
Bladder	0.00 ± 0.00	0.00 ± 0.00
Muscle	0.02 ± 0.01	0.01 ± 0.00
Fat	0.01 ± 0.00	0.00 ± 0.00
GI tract	0.35 ± 0.11	0.13 ± 0.05
Nontreated skin	0.07 ± 0.01	0.04 ± 0.01
	Percent of administered dose	
Blood[b]	0.03 ± 0.01	0.01 ± 0.00
Plasma[b,c]	0.02 ± 0.00	0.01 ± 0.00
RBCs[b,c]	0.01 ± 0.00	0.00 ± 0.00
Liver	0.11 ± 0.02	0.07 ± 0.02
Kidneys	0.01 ± 0.00	0.00 ± 0.00
Lungs	0.00 ± 0.00	0.00 ± 0.00
Brain	0.00 ± 0.00	0.00 ± 0.00
Spleen	0.00 ± 0.00	0.00 ± 0.00
Testes	0.00 ± 0.00	0.00 ± 0.00
Adrenals	0.00 ± 0.00	0.00 ± 0.00
Bladder	0.00 ± 0.00	0.00 ± 0.00
Muscle[b]	0.04 ± 0.01	0.02 ± 0.01
GI tract	0.18 ± 0.04	0.07 ± 0.02
Nontreated skin[b]	0.06 ± 0.01	0.04 ± 0.00
Urine	4.82 ± 0.42	4.00 ± 0.73
Feces	1.34 ± 0.14	1.06 ± 0.19
Dose wash	62.45 ± 2.62	77.92 ± 3.44
Application area	24.00 ± 4.38	8.93 ± 3.69
Recovery	93.02 ± 2.16	92.11 ± 2.08

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[a] Mean ± SE of three rats per group.

[b] Calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[c] Individual blood components and fat are not included in recovery estimates.

Table 20. Tissue-to-Blood Concentration Ratios from Male Fischer 344 Rats at 96 h Followig Dermal Treatment with ^{14}C-Labeled 4,4'-MDA (4.0 mg/Rat): Continuous Application for 96 h versus Skin Washing at 24 h[a]

Tissue	Continuous	24-h wash
Plasma	1.17	1.10
RBCs	0.65	0.60
Liver	6.42	8.68
Kidneys	2.35	2.10
Lungs	0.66	0.55
Brain	0.13	0.08
Spleen	0.38	0.33
Testes	0.46	0.38
Adrenals	1.84	1.18
Bladder	0.00	0.00
Muscle	0.27	0.23
Fat	0.09	0.05
GI tract	4.27	3.30
Nontreated skin	0.89	1.10

[a] Derived from mean of three rats per group.

Table 21. Recovery of Radioactivity in Blood, Tissue, and Excreta at 96 h Following Dermal Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (4.0 mg/Rat): Continuous Application for 96 h versus Skin Washing at 24 h[a]

	Percent of dose	
Tissue/excretum	Continuous	24-h wash
Blood	0.03 ± 0.01	0.01 ± 0.00
Tissue	0.22 ± 0.04	0.13 ± 0.02
GI tract	0.18 ± 0.04	0.07 ± 0.02
Urine	4.82 ± 0.42	4.00 ± 0.73
Feces	1.34 ± 0.14	1.06 ± 0.19
Total absorbed	6.58 ± 0.60	5.27 ± 0.96
Dose wash	62.45 ± 2.62	77.92 ± 3.44
Application area	24.00 ± 4.38	8.93 ± 3.69
Recovery	93.02 ± 2.16	92.11 ± 2.08

[a] Mean ± SE of three rats per group.

Table 22. Urinary and Fecal Excretion of Radioactivity in Male Hartley Guinea Pigs Treated Intravenously with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)[a]

Excretum	Time after dosing (h)	Percent of dose	Cumulative percent of dose
Urine	6	6.43 ± 0.86	6.43 ± 0.86
	12	15.33 ± 2.12	21.76 ± 2.16
	24	8.89 ± 2.04	30.65 ± 2.75
	48	3.31 ± 0.43	33.96 ± 2.97
	72	0.68 ± 0.05	34.64 ± 2.96
	96	0.35 ± 0.09	34.98 ± 3.02
Feces	6	1.78 ± 0.17	1.78 ± 0.17
	12	4.05 ± 2.06	5.83 ± 1.90
	24	27.29 ± 1.57	33.11 ± 2.94
	48	18.00 ± 2.45	51.11 ± 3.36
	72	4.07 ± 0.16	55.19 ± 3.45
	96	1.27 ± 0.28	56.45 ± 3.71
Total	6	8.21 ± 1.02	8.21 ± 1.02
	12	19.38 ± 3.54	27.58 ± 2.90
	24	36.18 ± 2.14	63.76 ± 0.78
	48	21.31 ± 2.85	85.07 ± 2.07
	72	4.75 ± 0.18	89.82 ± 1.95
	96	1.61 ± 0.19	91.43 ± 2.11

[a] Mean ± SE of three guinea pigs per group.

Table 23. Radioactivity in Blood, Tissue, and Excreta at 96 h Following Intravenous Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)[a]

Tissue/excretum	μg equivalents/g or mL	Percent of dose
Blood[b]	0.21 ± 0.03	0.55 ± 0.07
Plasma[b,c]	0.20 ± 0.03	0.31 ± 0.04
RBCs[b,c]	0.20 ± 0.01	0.21 ± 0.00
Liver	0.77 ± 0.08	1.68 ± 0.07
Kidneys	0.24 ± 0.04	0.11 ± 0.00
Lungs	0.39 ± 0.13	0.10 ± 0.04
Brain	0.00 ± 0.00	0.00 ± 0.00
Spleen	1.19 ± 0.10	0.06 ± 0.01
Testes	0.02 ± 0.01	0.00 ± 0.00
Adrenals	0.19 ± 0.02	0.00 ± 0.00
Bladder	0.01 ± 0.00	0.00 ± 0.00
Muscle[b]	0.01 ± 0.00	0.15 ± 0.04
Fat[c]	0.03 ± 0.00	-
GI tract	0.08 ± 0.02	0.61 ± 0.07
Skin[b]	0.05 ± 0.01	0.28 ± 0.02
Urine	-	34.98 ± 3.02
Feces	-	56.45 ± 3.71
Recovery	-	94.98 ± 2.20

[a] Mean ± SE of three guinea pigs per group.

[b] Percent of dose calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[c] Individual blood components and fat are not included in recovery estimates.

Table 24. Tissue-to-Blood Concentration Ratios from Male Hartley Guinea Pigs at 96 h Following Intravenous Treatment with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)[a]

Tissue	Ratio
Plasma	0.94
RBCs	0.96
Liver	3.60
Kidneys	1.15
Lungs	1.81
Brain	0.02
Spleen	5.57
Testes	0.10
Adrenals	0.90
Bladder	0.06
Muscle	0.05
Fat	0.13
GI tract	0.38
Skin	0.22

[a] Derived from mean of three guinea pigs per group.

Table 25. Recovery of Radioactivity in Blood, Tissue, and Excreta at 96 h Following Intravenous Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)[a]

Tissue/excretum	Percent of dose
Blood	0.55 ± 0.07
Tissue	2.39 ± 0.04
GI tract	0.61 ± 0.07
Urine	34.98 ± 3.02
Feces	56.45 ± 3.71
Recovery	94.98 ± 2.20

[a] Mean ± SE of three guinea pigs per group.

Table 26. Urinary and Fecal Excretion of Radioactivity in Male Hartley Guinea Pigs Treated Dermally with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)[a]

Excretum	Time after dosing (h)	Percent of dose 24-h sacrifice	 96-h sacrifice
Urine	6	2.03 ± 1.64	0.22 ± 0.05
	12	3.43 ± 1.54	1.30 ± 0.27
	24	2.35 ± 0.54	2.80 ± 0.76
	48		3.32 ± 0.75
	72		1.50 ± 0.40
	96		1.33 ± 0.44
Feces	6	0.05 ± 0.02	0.03 ± 0.01
	12	1.15 ± 0.30	0.77 ± 0.34
	24	4.50 ± 1.61	3.87 ± 0.58
	48		7.27 ± 1.24
	72		3.57 ± 0.58
	96		2.11 ± 0.28
Total	6	2.08 ± 1.66	0.25 ± 0.06
	12	4.58 ± 1.84	2.07 ± 0.10
	24	6.85 ± 2.12	6.67 ± 0.32
	48		10.59 ± 1.88
	72		5.07 ± 0.98
	96		3.44 ± 0.67
		Cumulative percent of dose	
Urine	6	2.03 ± 1.64	0.22 ± 0.05
	12	5.45 ± 2.10	1.52 ± 0.30
	24	7.81 ± 2.52	4.32 ± 0.86
	48		7.64 ± 1.51
	72		9.13 ± 1.89
	96		10.47 ± 2.21
Feces	6	0.05 ± 0.02	0.03 ± 0.01
	12	1.20 ± 0.31	0.80 ± 0.34
	24	5.70 ± 1.89	4.67 ± 0.80
	48		11.94 ± 0.63
	72		15.52 ± 1.16
	96		17.62 ± 1.40
Total	6	2.08 ± 1.66	0.25 ± 0.06
	12	6.65 ± 2.35	2.32 ± 0.05
	24	13.51 ± 3.91	8.99 ± 0.36
	48		19.58 ± 1.96
	72		24.65 ± 2.93
	96		28.09 ± 3.45

[a] Mean ± SE of three guinea pigs per group.

Table 27. Radioactivity in Blood, Tissue, and Excreta at 6, 24, and 96 h Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)[a]

Tissue/excretum	μg equivalents/g or mL		
	6 h	24 h	96 h
Blood	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Plasma	0.05 ± 0.00	0.06 ± 0.01	0.06 ± 0.01
RBCs	0.03 ± 0.00	0.04 ± 0.01	0.06 ± 0.01
Liver	0.25 ± 0.02	0.28 ± 0.04	0.19 ± 0.05
Kidneys	0.09 ± 0.00	0.12 ± 0.02	0.11 ± 0.03
Lungs	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
Brain	0.03 ± 0.01	0.02 ± 0.00	0.00 ± 0.00
Spleen	0.04 ± 0.00	0.03 ± 0.01	0.02 ± 0.01
Testes	0.12 ± 0.02	0.07 ± 0.02	0.02 ± 0.00
Adrenals	1.10 ± 0.40	0.98 ± 0.03	0.51 ± 0.08
Bladder	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
Muscle	0.02 ± 0.01	0.01 ± 0.00	0.03 ± 0.02
Fat	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
GI tract	0.28 ± 0.01	0.67 ± 0.16	0.10 ± 0.03
Nontreated skin	0.05 ± 0.01	0.05 ± 0.02	0.04 ± 0.02
	Percent of administered dose		
Blood[b]	0.12 ± 0.02	0.14 ± 0.03	0.14 ± 0.02
Plasma[b,c]	0.08 ± 0.01	0.09 ± 0.02	0.08 ± 0.02
RBCs[b,c]	0.02 ± 0.00	0.04 ± 0.01	0.06 ± 0.01
Liver	0.40 ± 0.01	0.48 ± 0.07	0.40 ± 0.08
Kidneys	0.04 ± 0.00	0.04 ± 0.01	0.05 ± 0.01
Lungs	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Brain	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Spleen	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Testes	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Adrenals	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
Bladder	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Muscle[b]	0.31 ± 0.10	0.19 ± 0.04	0.37 ± 0.25
GI tract	1.51 ± 0.11	2.80 ± 0.95	0.59 ± 0.14
Nontreated skin[b]	0.30 ± 0.07	0.27 ± 0.12	0.23 ± 0.09
Urine	0.35 ± 0.10	7.81 ± 2.52	10.47 ± 2.21
Feces	0.10 ± 0.03	5.70 ± 1.89	17.62 ± 1.40
Dose wash	80.55 ± 1.49	58.78 ± 7.31	40.85 ± 4.30
Application area	11.43 ± 1.08	14.77 ± 1.73	29.41 ± 2.31
Recovery	95.16 ± 2.56	91.01 ± 1.44	100.14 ± 2.69

[a] Mean ± SE of three guinea pigs per group.

[b] Calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[c] Individual blood components and fat are not included in recovery estimates.

Table 28. Tissue-to-Blood Concentration Ratios from Male Hartley Guinea Pigs at 6, 24, or 96 h Following Dermal Treatment with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)[a]

Tissue	6 h	24 h	96 h
Plasma	1.08	1.02	0.95
RBCs	0.52	0.71	0.97
Liver	5.25	4.71	3.29
Kidneys	1.92	2.10	1.83
Lungs	1.10	0.95	0.60
Brain	0.63	0.31	0.07
Spleen	0.73	0.44	0.29
Testes	2.44	1.10	0.41
Adrenals	22.88	16.54	8.86
Bladder	0.13	0.03	0.09
Muscle	0.46	0.24	0.45
Fat	0.60	0.24	0.19
GI tract	5.73	11.39	1.79
Nontreated skin	1.13	0.83	0.72

[a] Derived from mean of three guinea pigs per group.

Table 29. Recovery of Radioactivity in Blood, Tissue, and Excreta at 6, 24, or 96 h Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)

Tissue/excretum	Percent of dose		
	6 h	24 h	96 h
Blood	0.12 ± 0.02	0.14 ± 0.03	0.14 ± 0.02
Tissue	1.11 ± 0.17	1.02 ± 0.24	1.08 ± 0.29
GI tract	1.51 ± 0.11	2.80 ± 0.95	0.59 ± 0.14
Urine	0.35 ± 0.10	7.81 ± 2.52	10.47 ± 2.21
Feces	0.10 ± 0.03	5.70 ± 1.89	17.62 ± 1.40
Total absorbed	3.19 ± 0.18	17.46 ± 4.81	29.89 ± 3.72
Dose wash	80.55 ± 1.49	58.78 ± 7.31	40.85 ± 4.30
Application area	11.43 ± 1.08	14.77 ± 1.73	29.41 ± 2.31
Recovery	95.16 ± 2.56	91.01 ± 1.44	100.14 ± 2.69

[a] Mean ± SE of three guinea pigs per group.

Table 30. Urinary and Fecal Excretion of Radioactivity in Male Hartley Guinea Pigs Treated Dermally with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig): Application Area Washed at 24 h[a]

Excretum	Time after dosing (h)	Percent of dose	Cumulative percent of dose
Urine	6	0.17 ± 0.03	0.17 ± 0.03
	12	1.53 ± 0.22	1.70 ± 0.21
	24	2.28 ± 0.15	3.99 ± 0.36
	48	2.12 ± 0.42	6.11 ± 0.52
	72	0.26 ± 0.04	6.37 ± 0.50
	96	0.19 ± 0.03	6.56 ± 0.48
Feces	6	0.02 ± 0.01	0.02 ± 0.01
	12	1.14 ± 0.10	1.16 ± 0.11
	24	3.99 ± 0.99	5.15 ± 0.95
	48	4.94 ± 0.34	10.09 ± 0.61
	72	0.50 ± 0.01	10.59 ± 0.60
	96	0.30 ± 0.07	10.89 ± 0.59
Total	6	0.20 ± 0.03	0.20 ± 0.03
	12	2.67 ± 0.13	2.87 ± 0.10
	24	6.27 ± 1.06	9.14 ± 1.14
	48	7.06 ± 0.76	16.20 ± 0.61
	72	0.76 ± 0.04	16.96 ± 0.57
	96	0.49 ± 0.11	17.45 ± 0.48

[a] Mean ± SE of three guinea pigs per group.

Table 31. Radioactivity in Blood, Tissue, and Excreta Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig): Nonoccluded Skin and Skin Washed at 24 h[a]

	µg equivalents/g or mL	
Tissue/excretum	Nonoccluded/6-h sacrifice	24-h wash/96-h sacrifice
Blood	0.05 ± 0.00	0.03 ± 0.00
Plasma	0.05 ± 0.00	0.03 ± 0.00
RBCs	0.03 ± 0.01	0.04 ± 0.00
Liver	0.22 ± 0.02	0.11 ± 0.01
Kidneys	0.19 ± 0.06	0.06 ± 0.00
Lungs	0.05 ± 0.00	0.02 ± 0.00
Brain	0.03 ± 0.00	0.00 ± 0.00
Spleen	0.04 ± 0.00	0.01 ± 0.00
Testes	0.10 ± 0.01	0.01 ± 0.00
Adrenals	0.83 ± 0.13	0.07 ± 0.00
Bladder	0.01 ± 0.00	0.00 ± 0.00
Muscle	0.02 ± 0.00	0.00 ± 0.00
Fat	0.02 ± 0.00	0.01 ± 0.00
GI tract	0.20 ± 0.05	0.02 ± 0.00
Nontreated skin	0.04 ± 0.01	0.01 ± 0.00
	Percent of administered dose	
Blood[b]	0.12 ± 0.02	0.08 ± 0.00
Plasma[b,c]	0.07 ± 0.01	0.04 ± 0.00
RBCs[b,c]	0.03 ± 0.01	0.04 ± 0.00
Liver	0.37 ± 0.07	0.22 ± 0.03
Kidneys	0.09 ± 0.02	0.02 ± 0.00
Lungs	0.01 ± 0.00	0.00 ± 0.00
Brain	0.01 ± 0.00	0.00 ± 0.00
Spleen	0.00 ± 0.00	0.00 ± 0.00
Testes	0.01 ± 0.00	0.00 ± 0.00
Adrenals	0.01 ± 0.00	0.00 ± 0.00
Bladder	0.00 ± 0.00	0.00 ± 0.00
Muscle[b]	0.26 ± 0.04	0.04 ± 0.01
GI tract	1.27 ± 0.30	0.12 ± 0.04
Nontreated skin[b]	0.25 ± 0.05	0.05 ± 0.01
Urine	0.60 ± 0.47	6.56 ± 0.48
Feces	0.12 ± 0.06	10.89 ± 0.59
Dose wash	87.46 ± 1.24	61.78 ± 1.06
Application area	9.37 ± 0.61	16.58 ± 1.34
Recovery	99.97 ± 1.04	96.34 ± 0.61

[a] Mean ± SE of three guinea pigs per group.

[b] Calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[c] Individual blood components and fat are not included in recovery estimates.

Table 32. Tissue-to-Blood Concentration Ratios from Male Hartley Guinea Pigs Following Dermal Treatment with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig): Nonoccluded Skin and Skin Washed at 24 h[a]

Tissue	Nonoccluded/6-h sacrifice	24-h wash/96-h sacrifice
Plasma	1.02	0.76
RBCs	0.71	1.15
Liver	4.96	3.27
Kidneys	4.22	1.76
Lungs	1.16	0.48
Brain	0.67	0.06
Spleen	0.87	0.27
Testes	2.20	0.15
Adrenals	18.44	2.00
Bladder	0.16	0.09
Muscle	0.40	0.09
Fat	0.33	0.24
GI tract	4.36	0.55
Nontreated skin	0.93	0.27

[a] Derived from mean of three guinea pigs per group.

Table 33. Summary of Radioactivity in Blood, Tissue, and Excreta Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig): Nonoccluded Skin and Skin Washed at 24-h[a]

	Percent of dose	
Tissue/excretum	Nonoccluded/6-h sacrifice	24-h wash/96-h sacrifice
Blood	0.12 ± 0.02	0.08 ± 0.00
Tissue	1.02 ± 0.05	0.33 ± 0.03
GI tract	1.27 ± 0.30	0.12 ± 0.04
Urine	0.60 ± 0.47	6.56 ± 0.48
Feces	0.12 ± 0.06	10.89 ± 0.59
Total absorbed	3.14 ± 0.56	17.98 ± 0.48
Dose wash	87.46 ± 1.24	61.78 ± 1.06
Application area	9.37 ± 0.61	16.58 ± 1.34
Recovery	99.97 ± 1.04	96.34 ± 0.61

[a] Mean ± SE of three guinea pigs per group.

Table 34. Urinary and Fecal Excretion of Radioactivity in Male Hartley Guinea Pigs Treated Dermally with ^{14}C-Labeled 4,4'-MDA (10.0 mg/Guinea Pig): Continuous Application for 96 h versus Washing at 24 h

Excretum	Time after dosing (h)	Percent of dose	
		Continuous[a]	24-h wash[b]
Urine	6	0.04	0.05 ± 0.03
	12	0.26	0.16 ± 0.01
	24	0.24	0.29 ± 0.03
	48	0.40	0.60 ± 0.21
	72	0.68	0.26 ± 0.09
	96	1.13	0.19 ± 0.06
Feces	6	0.01	0.00 ± 0.00
	12	0.03	0.06 ± 0.00
	24	0.70	0.52 ± 0.05
	48	0.97	0.78 ± 0.18
	72	0.85	0.43 ± 0.15
	96	1.06	0.39 ± 0.13
Total	6	0.05	0.05 ± 0.03
	12	0.29	0.21 ± 0.02
	24	0.94	0.80 ± 0.08
	48	1.37	1.38 ± 0.38
	72	1.54	0.69 ± 0.21
	96	2.19	0.58 ± 0.19
		Cumulative percent of dose	
Urine	6	0.04	0.05 ± 0.03
	12	0.30	0.20 ± 0.02
	24	0.54	0.49 ± 0.03
	48	0.94	1.09 ± 0.18
	72	1.63	1.35 ± 0.25
	96	2.75	1.54 ± 0.30
Feces	6	0.01	0.00 ± 0.00
	12	0.04	0.06 ± 0.00
	24	0.74	0.58 ± 0.05
	48	1.70	1.35 ± 0.13
	72	2.56	1.78 ± 0.27
	96	3.62	2.18 ± 0.39
Total	6	0.05	0.05 ± 0.03
	12	0.34	0.26 ± 0.01
	24	1.28	1.07 ± 0.08
	48	2.65	2.45 ± 0.30
	72	4.18	3.14 ± 0.51
	96	6.37	3.72 ± 0.69

[a] Average of two guinea pigs.
[b] Mean ± SE of three guinea pigs.

Table 35. Radioactivity in Blood, Tissue, and Excreta at 96 h Following Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (10.0 mg/Guinea Pig): Continuous Application for 96 h versus Washing at 24 h

	µg equivalents/g or mL	
Tissue/excretum	Continuous[a]	24-h wash[b]
Blood	0.16	0.09 ± 0.03
Plasma	0.17	0.09 ± 0.03
RBCs	0.12	0.08 ± 0.02
Liver	0.59	0.26 ± 0.09
Kidneys	0.31	0.15 ± 0.06
Lungs	0.12	0.03 ± 0.01
Brain	0.01	0.01 ± 0.00
Spleen	0.04	0.01 ± 0.00
Testes	0.43	0.03 ± 0.01
Adrenals	2.18	0.72 ± 0.38
Bladder	0.01	0.02 ± 0.01
Muscle	0.04	0.02 ± 0.01
Fat	0.06	0.01 ± 0.00
GI tract	0.89	0.41 ± 0.17
Nontreated skin	0.40	0.08 ± 0.02
	Percent of administered dose	
Blood[c]	0.04	0.02 ± 0.01
Plasma[c,d]	0.03	0.01 ± 0.00
RBCs[c,d]	0.01	0.01 ± 0.00
Liver	0.13	0.05 ± 0.02
Kidneys	0.01	0.01 ± 0.00
Lung	0.00	0.00 ± 0.00
Brain	0.00	0.00 ± 0.00
Spleen	0.00	0.00 ± 0.00
Testes	0.00	0.00 ± 0.00
Adrenals	0.00	0.00 ± 0.00
Bladder	0.00	0.00 ± 0.00
Muscle[c]	0.05	0.03 ± 0.02
GI tract	0.64	0.28 ± 0.11
Nontreated skin[c]	0.22	0.04 ± 0.01
Urine	2.75	1.54 ± 0.30
Feces	3.62	2.18 ± 0.39
Dose wash	69.70	80.44 ± 2.44
Application area	13.72	7.29 ± 1.36
Recovery	90.89	91.88 ± 1.40

[a] Average of two guinea pigs.
[b] Mean ± SE of three guinea pigs.
[c] Calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.
[d] Individual blood components and fat are not included in recovery estimates.

Table 36. Tissue-to-Blood Concentration Ratios from Male Hartley Guinea Pigs at 96 h Following Dermal Treatment with ^{14}C-Labeled 4,4'-MDA (10.0 mg/Guinea Pig): Continuous Application for 96 h versus Washing at 24 h

Tissue	Continuous[a]	24-h wash[b]
Plasma	1.10	0.98
RBCs	0.79	0.89
Liver	3.75	2.91
Kidneys	1.99	1.71
Lungs	0.75	0.29
Brain	0.06	0.06
Spleen	0.27	0.16
Testes	2.75	0.38
Adrenals	13.85	8.13
Bladder	0.05	0.22
Muscle	0.23	0.22
Fat	0.36	0.12
GI tract	5.64	4.58
Nontreated skin	2.55	0.84

[a] Derived from average of two guinea pigs.
[b] Derived from mean of three guinea pigs.

Table 37. Recovery of Radioactivity in Blood, Tissue, and Excreta at 96 h Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (10.0 mg/Guinea Pig): Continuous Application for 96 h versus Washing at 24 h

	Percent of dose	
Tissue/excretum	Continuous[a]	24-h Wash[b]
Blood	0.04	0.02 ± 0.01
Tissue	0.43	0.13 ± 0.04
GI tract	0.64	0.28 ± 0.11
Urine	2.75	1.54 ± 0.30
Feces	3.62	2.18 ± 0.39
Total absorbed	7.48	4.15 ± 0.81
Dose wash	69.70	80.44 ± 2.44
Application area	13.72	7.29 ± 1.36
Recovery	90.89	91.88 ± 1.40

[a] Average of two guinea pigs.
[b] Mean ± SE of three guinea pigs.

Table 38. Urinary and Fecal Excretion of Radioactivity in Male Rhesus Monkeys Treated Dermally or Intravenously with ^{14}C-Labeled 4,4'-MDA (10.0 mg/Monkey)[a]

Excretum	Time after dosing (h)	Percent of dose	
		Dermal	Intravenous
Urine	6	0.19 ± 0.14	24.85 ± 6.70
	12	0.81 ± 0.20	21.10 ± 10.84
	24	2.84 ± 1.25	17.74 ± 1.60
	48	7.36 ± 1.36	15.66 ± 10.04
	72	3.92 ± 1.26	3.13 ± 1.76
	96	1.64 ± 0.50	0.78 ± 0.28
	120	0.90 ± 0.28	0.37 ± 0.06
	144	0.64 ± 0.20	0.27 ± 0.06
	168	0.53 ± 0.18	0.44 ± 0.20
Feces	6	0.00[b]	0.07 ± 0.05
	12	0.00[c]	0.06[c]
	24	0.15 ± 0.12	3.93 ± 2.01
	48	0.39 ± 0.29	2.52 ± 0.87
	72	0.47 ± 0.04	2.37 ± 1.60
	96	0.37 ± 0.15	0.39 ± 0.22
	120	0.15 ± 0.04	0.18 ± 0.04
	144	0.28 ± 0.17	0.16 ± 0.04
	168	0.12 ± 0.05	0.11 ± 0.01
		Cumulative percent of dose	
Urine	6	0.19 ± 0.14	24.85 ± 6.70
	12	1.01 ± 0.32	45.95 ± 10.99
	24	3.84 ± 1.55	63.69 ± 11.45
	48	11.20 ± 1.42	79.35 ± 1.62
	72	15.12 ± 1.73	82.48 ± 1.14
	96	16.75 ± 2.09	83.27 ± 1.32
	120	17.66 ± 2.31	83.64 ± 1.38
	144	18.30 ± 2.50	83.90 ± 1.41
	168	18.83 ± 2.58	84.34 ± 1.57
Feces	6	0.00[b]	0.07 ± 0.05
	12	0.00[b]	0.09 ± 0.07
	24	0.15 ± 0.12	4.02 ± 1.95
	48	0.55 ± 0.41	6.54 ± 2.81
	72	1.02 ± 0.41	8.92 ± 1.28
	96	1.39 ± 0.27	9.31 ± 1.09
	120	1.54 ± 0.23	9.49 ± 1.08
	144	1.81 ± 0.21	9.65 ± 1.12
	168	1.93 ± 0.22	9.75 ± 1.12
Dose wash		47.27 ± 5.90	-
Recovery		68.03 ± 3.24	94.09 ± 0.97

[a] Mean ± SE of three monkeys per group.
[b] Average of two monkeys.
[c] One monkey.

APPENDIX I

STUDY PROTOCOL

Midwest Research Institute
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Kansas City, Missouri 64110

MRI Project No. 7901-A(21)
June 26, 1984

Study Protocol

DERMAL PENETRATION MODEL STUDIES: DERMAL ABSORPTION OF ^{14}C-LABELED METHYLENEDIANILINE (MDA) IN RATS, GUINEA PIGS, AND MONKEYS

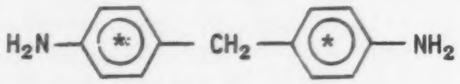
Prepared for

U.S. Environmental Protection Agency
Office of Toxic Substances
Field Studies Branch, TS-798
Washington, DC 20460

A. Objectives

The overall objective of this study is to develop dermal penetration data from representative chemical models that can be used in prediction of dosage from exposure to toxic chemicals. The specific aims are to select and compare techniques and animal models for measuring dermal penetration rates of a selected model chemical. The studies will be performed *in vivo* with three animal models: the rat, guinea pig, and monkey.

The chemical selected is 4,4'-methylene dianiline (MDA, I.), a representative aromatic amine with known toxic and carcinogenic properties.



I. 4,4'-Methylene dianiline (MDA)
(* The position of the ^{14}C-label)

Several experimental conditions will be considered and assessed in the study protocol include skin type (rat, guinea pig, and monkey), dosage (low, high), exposure regimen (limited, continuous), occlusion (exposed, covered) and carrier. In addition, initial studies will be performed to assess the surface/dermal washing efficiency following application to rats, guinea pigs, and monkeys.

Limited experiments will be performed to assess the elimination of MDA following intravenous (i.v.) administration to utilize the data generated in calculating the absolute absorption of MDA following dermal application. The utility of this methodology will be assessed by comparing the data generated to those resulting from adding of excretory data in urine and feces of animals exposed to the dermal application.

The species selected are the rat, a species for which historical data on the toxicity and carcinogenicity of aromatic amines are available and which is used extensively in dermal absorption studies, the guinea pig and the monkey, species which have skin characteristics that resemble human skin. The studies in rats and guinea pigs will be performed at two dose levels, "low" and "high". The monkey studies will be carried out with the low dose only.

B. Animals

Adult male Fischer 344 rats, Hartley guinea pigs, and Rhesus monkeys will be used in the studies. The rats 7 to 9 weeks old and weighing 125 to 175 g, and the guinea pigs, 5 to 7 weeks old and weighing 400 to 500 g, will be purchased from Charles River Breeding Laboratories, North Wilmington, Massachusetts. The monkeys, $\sim$ 4 yr old and weighing $\sim$ 5 kg, will be purchased from Charles River Research Primates Corporation, Port Washington, New York. Upon arrival, the rats and guinea pigs will be identified by metal ear tags and the monkeys will be tattooed across the chest. Prior to testing, the animals will be selected at random for each group. Animals showing signs of ill health will not be used.

C. Animal Care

Animal care and housing will be in accordance with DHEW Publication No. (NIH)-78-23, 1978, "Guidelines for the Care and Use of Laboratory Animals," and the MRI Manual for Animal Care.

The animals will be housed in environmentally controlled rooms with 10 to 15 air changes per hour. The rooms will be maintained at a temperature of 25 ± 2°C and humidity of 50 ± 10%, with a 12-hr light/dark cycle per day. The rats and guinea pigs will be kept in the quarantine facility for at least 7 days prior to use. Monkeys will be acclimatized for a minimum of 3 weeks and will be subjected to T.B. tests on arrival and 2 weeks later. The attendant veterinarian will examine the animals prior to release for the studies.

During the acclimation period, the rats and guinea pigs will be housed in polycarbonate cages on Ab-Sorb-Dri® hardwood chip bedding, and the monkeys will be housed in stainless steel cages. All animals will be provided with certified feed and tap water _ad libitum_. The guinea pigs diet will be supplemented with appropriate amounts of ascorbic acid in the drinking water, and the monkey diet will be supplemented with oranges. There are no known contaminants in the food or water that would interfere with the study.

D. Test Compound

^{14}C-labeled MDA will be synthesized by the BioOrganic Chemistry Department of MRI. About 10 mCi of ring-^{14}C-labeled MDA will be synthesized with specific activity of > 10 mCi/mmol. Information on the identity, purity, and stability of the ^{14}C-labeled compound will be supplied. Nonlabeled MDA will be purchsed from Aldrich Chemical Company. Identity and purity of the nonlabeled compound will be checked by TLC. The nonlabeled and ^{14}C-labeled test compounds will be stored under specific conditions which minimize decomposition.

E. Dosage and Treatment

Two dose levels will be used in the study, a "low" dose (2 mg/kg) and a "high" dose (20 mg/kg). A mixture of the ^{14}C-labeled and nonlabeled MDA will be dissolved in ethanol:water mixture and applied to the skin or administered intravenously (i.v.) to rats, guinea pigs, and monkeys. For the dermal studies, both the low and high doses will contain ~ 200 µCi/kg (~ 30 µCi/rat, ~ 100 µCi/guinea pig, and ~ 1,000 µCi/monkey). For the i.v. studies, the low dose will contain ~ 100 µCi/kg (~ 15 µCi/rat, ~ 50 µCi/guinea pig, and ~ 500 µCi/monkey).

(95:5)

mEh For dermal treatment, the doses will be administered in a mixture of ethanol:water (~~50:50~~) and applied at a volume of 0.5 mL/kg (0.1 mL/rat, 0.25 mL/guinea pig, and 2.5 mL/monkey). The backs of the rats and guinea pigs, and the lateral forearm of the monkeys will be lightly shaved with an electric clipper shortly before treatment. The dose will be applied with a disposable micropipette on a specific area (~ 2 cm² for rats, ~ 5 cm² for guinea pigs, and ~ 50 cm² for monkeys) on the freshly shaven skin. Except when indicated otherwise (see Study Design), the dosed areas will be occluded with an aluminum foil patch which will be secured in place with adhesive tape.

For intravenous treatment, a mixture of the ^{14}C-labeled and nonlabeled MDA will be dissolved in ethanol:water (25:75) and administered at the low dose level in a volume of 1.0 mL/kg (0.2 mL for rats, 0.5 mL/kg for guinea pigs, and 5 mL for monkeys). Intravenous dosing for the three species will be performed through the saphenous vein.

F. Study Design (See Table 1)

1. Preliminary Studies

These studies will be performed with 3 male rats and 3 male guinea pigs treated dermally with ^{14}C-MDA at the low or high dose levels, or i.v. at the low dose level (1 animal per treatment). The animals will be housed in individual metabolic cages for collection of urine and feces at 6, 12, 24, 48, 72, and 96 hr following dosing. Radioactivity remaining on the skin of dermally treated animals will be washed with soap and water or acetone and water (see below) then the animals will be sacrificed for sampling of selected tissues. These studies will assist in selecting the appropriate sampling times for the definitive experiments described below and in testing the method of treatments for rats and guinea pigs.

2. Washing Efficiency Studies

Before initiation of the dermal disposition studies described below, an initial washing efficiency experiment will be performed to assess the extent of removal of the applied ^{14}C-labeled material by washing with soap and water, or organic solvents. Six rats, six guinea pigs, and four monkeys will be lightly anesthetized with sodium pentobarbital then treated with dermal doses of ^{14}C-MDA at the low dose level. Immediately following application (5 to 10 min) the doses will be washed with soap and water or with acetone and water (3 rats, 3 guinea pigs, 2 monkeys for each treatment) then the animals housed in individual metabolic cages for excreta collection. Urine and feces will be collected at 6, 12, 24, and 48 hr following dosing. Collection of excreta may continue every 24 hr if significant amounts of radioactivity continue to be eliminated. Determination of dermal washing efficiency at other times following application (6, 24, and 96 hr) will be assessed in the absorption studies described below.

3. Rat Studies

These studies will be performed with 21 rats treated dermally at the low or high dose levels and 9 rats treated i.v. at the low dose level. The dermal dose of ^{14}C-MDA will be applied for 6 or 24 hr then removed or kept on the skin for the duration of the study (96 hr). After application, the animals will be placed in metabolic cages for excreta collection. Urine and feces will be collected at 6, 12, 24, 48, 72, and 96 hr. At the specified times, the applied doses will be washed with soap and water or acetone and water as determined from the washing efficiency studies, then the animals sacrificed (6 and 24 hr) or returned to the metabolic cages. A group of 3 rats will be treated dermally at the low dose level and placed in individual restrainers for 6 hr (until sacrifice). In this group, the dosing area will be kept uncovered to determine the effect of nonocclusion on the dermal penetration of ^{14}C-MDA.

In the i.v. studies the rats will be treated with the low dose of ^{14}C-MDA and placed in individual metabolic cages for collection of excreta at 6, 12, 24, 48, 72, and 96 hr following administration. Three animals will be sacrificed at 6, 24, or 96 hr for tissue sampling.

4. Guinea Pig Studies

These studies will be performed with 21 male guinea pigs treated dermally at 2 dose levels and 3 guinea pigs treated i.v. The design will be similar to the rat studies except that i.v. dosing will be limited to only 3 animals which will be treated with the low dose and sacrificed at 96 hr following dosing.

5. Monkey Studies

In these studies, 4 male monkeys will be treated dermally (2), or i.v. (2) with the low dose of ^{14}C-MDA. The monkeys will be acclimated for two 24-hr periods in metabolic charis during the week perior to dosing. After dermal application the monkeys will be placed in metabolic chairs for the first 24 hr. The doses will be removed, washed with soap and water or acetone and water and the animals will be transferred to individual metabolic cages for the remainder of the studies. Following application, urine and feces will be collected at 6, 12, 24, 48, 72 and 96 hr following dosing. The monkeys treated i.v. will also be placed in the metabolic chairs for 24 hr then in metabolic cages for the remainder of the study. Collection of excreta will performed similarly.

The data collected following dermal application will be compared to those obtained following i.v. administration of ^{14}C-MDA. Selected tissues may be sampled at the end of the study (96 hr) or the monkeys will be retained for future investigations with ^{14}C-MDA or other test chemicals. Crossover studies can be considered if the data obtained from the 4 monkeys show significant variability. However, the persistence of radioactivity in blood or tissue components e.g., hemoglobin, may hinder the reutilization of these animals except following extended periods after treatment.

G. Sample Collection

Urine will be collected in containers kept on dry ice. After each collection, the cages will be rinsed and the cage washings will be measured and analyzed. At sacrifice, all animals will be anesthetized by injection of sodium pentobarbital and exsanguinated by withdrawal of blood from appropriate sites. The application areas will be washed with a suitable solvent and the washings will be measured and analyzed. The following tissues and organs will be removed, washed with saline, blotted with absorbing paper, weighed, and prepared for radiochemical analyses:

- Liver
- Kidneys
- Lungs
- Brain
- Spleen
- Adrenals
- Testes
- Urinary bladder
- GI Tract plus contents
- Skeletal muscle
- Retroperitoneal fat
- Skin from nontreated areas
- Application area

Portions of blood will be centrifuged to separate plasma and red blood cells (RBCs). Bladder contents will be removed and the bladder will be washed thoroughly with saline. The contents and washings will be combined with the final urine samples and analyzed. Blood and tissue will be kept on ice during the necropsy procedures. Sample preparation and analyses will be performed immediately after collection, or the samples will be frozen on dry ice and stored frozen until analyzed. If requested by the project monitor, tissue and excreta will be stored for future metabolic studies.

H. Sample Preparation and Analysis

Blood, tissue, and excreta will be analyzed in duplicate whenever possible. Assays not within ± 10% of the mean of the duplicates will be reassayed except when no more sample is available or when radioactivity counts are low and nonsignificant, i.e., less than two times the background.

Aliquots of the whole blood, RBCs and plasma will be analyzed for total radioactivity determination. Volumes of urine and cage rinse will be measured, and samples will be counted. Feces, GI tract (plus contents), and tissues weighing more than 0.25 g will be homogenized in an appropriate solvent (e.g., ethanol:water, 10:90). Aliquots of the homogenates will be measured and analyzed. Fat and nontreated skin samples will be weighed and assayed for ^{14}C content. The application areas of the skin and the covers will be soaked in a suitable solvent (e.g., acetone:water), and the extracted radioactivity will be analyzed. Organs and tissues (weighing less than 0.25 g) will be weighed and assayed for ^{14}C content. Blood, tissue, and fecal samples will be combusted using a Packard Tricarb Oxidizer Model C306. Counting will be performed in a Packard (Model 3255 or 4530) Tricarb liquid scintillation counter. Correction for background and efficiency will be performed.

I. Data Processing, Analysis, and Reporting

Appropriate methods will be used to present the ^{14}C contents of blood, tissues, and excreta in terms of microgram equivalents per gram of tissue or milliliter of fluid and/or percentage of the dose administered to each animal. Cumulative excretion in urine and feces will be calculated. The data from each treatment group will be expressed as mean ± S.E.

When applicable, statistical analyses between groups will be compared using a one-way analysis of variance. The differences between groups will be assessed either parametrically (Student's t-test) or nonparametrically (Mann-Whitney U test). In both cases, the data will be expressed as means ± S.E. and a p value of equal or less than 0.05 will be considered significant.

A final comprehensive report will be prepared describing, in detail, the methods used and the data generated from the study. The data will be summarized in tabular and/or graphical form.

J. Quality Assurance

Quality assurance of the data generated on the program will be monitored both within the program and externally by MRI's Quality Assurance Unit (QAU). All testing will be in accordance with the EPA Good Laboratory Practices Standards of November 29, 1983 (Federal Register, 48, 53922-53944. Conformity to the GLPs will be monitored by the QAU. After completion of the project, all raw data will be archived at MRI and will be available for inspection upon request. (See Appendix A for the Quality Assurance Program.)

K. Personnel Safety

The general safety policies of the Institute are established and enforced by the Institute Safety Committee, the Carcinogen Safety Committee, and the Radiation Safety Committee. It is anticipated that any nonroutine hazards associated with the use of the test compound will be identified in a project-specific health and safety plan. (See Appendix B for details of Safety Plan.)

L. Study Personnel

The studies will be conducted in the Chemical and Biological Sciences Department, Dr. James L. Spigarelli, Director. Dr. John Going, Project Manager, will provide administrative oversight for the program. Dr. Monaem El-hawari, Principal Toxicologist, will serve as the Study Director and will be responsible for the technical aspects of the program. Ms. Maxine Stoltz, Associate Biochemist, will supervise the animal studies and will be assisted by Mr. Steve Unwin, Associate Biologist, and Ms. Patricia Alm, Technician. Mr. Edward Williams will supervise the animal care activities under the supervision of Dr. Clifford Templeman, Veterinarian. Dr. Eugene Podrebarac, Manager, Quality Assurance will provide QA oversight for the program. Resumés of the key technical personnel are attached (see Appendix C).

M. Study Schedule (See Table 2)

Proposed Starting Date: July 9, 1984

Proposed Completion Date: October 22, 1984

Draft Final Report: November 12, 1984

Table 1. ^{14}C-MDA Study Design[a,b,c]

Species (strain)	Number	Route	Dose (mg/kg)	Application period	Number of animals per sacrifice[d,e]		
					6 hr	24 hr	96 hr
Rat (Fischer)	30	Dermal	2 "low"	6/24 hr	3 + 3	3[g]	3[g]
				Continuous	-	-	3
		Dermal	20 "high"	24 hr	-	-	3
				Continuous	-	-	3
		i.v.	2 "low"	Bolus	3	3	3
Guinea pig (Hartly)	24	Dermal	2 "low"	6/24 hr	3 + 3[f]	3[g]	3[g]
				Continuous	-	-	3
		Dermal	20 "high"	24 hr	-	-	3[g]
				Continuous	-	-	3
		i.v.	2 "low"	Bolus	-	-	3
Monkeys (Rhesus)	4	Dermal	2 "low"	24 hr	-	-	2[g]
		i.v.	2 "low"	Bolus	-	-	2

[a] A preliminary absorption study will be performed with 3 rats and 3 guinea pigs which will be treated with ^{14}C-MDA dermally at the low or high dose or intravenously at the low dose level (1 each treatment). Urine and feces will be collected at 6, 12, 24, 48, 72, and 96 hr when the animals will be sacrificed for tissue sampling.

[b] A dermal washing efficiency study will be performed with 6 rats, 6 guinea pigs, and 4 monkeys which will be treated with the low dose of ^{14}C-MDA then the dose washed immediately (5 to 10 min after application) with soap and water (half of each group) or with acetone and water (the other half). After washing the animals will be housed in individual metabolic cages for excreta collection at 6, 12, 24, and 48 hr.

[c] Details of the monkey studies design will be determined based on the data obtained from the rats and guinea pigs studies and on the information generated during the dermal washing efficiency experiments.

[d] For all animals kept beyond 6 hr, urine and feces will be collected at 6, 12, 24, 48, 72, and 96 hr after dosing.

[e] At the specified times, rats and guinea pigs will be sacrificed for tissue sampling. Collection of tissues from monkeys will be determined based on data generated from the rats and guinea pigs.

[f] Groups of 3 rats and 3 guinea pigs will be exposed dermally to the low dose of ^{14}C-MDA and kept restrained with the skin application area nonoccluded until sacrifice (6 hr). For all other rats and guinea pigs treated dermally, the application area will be covered (see text) and the animals will be housed in metabolic cages for excreta collection.

[g] For these groups of animals, the dermal dose will be removed at 24 hr following application. For the other groups, the dermal dose will be kept on the skin for the duration of the study.

N. Protocol Approvals

Mnaem El-Lawari	5/27/84	Janet C. Remmers	7/16/84
Study Director/Task Leader (MRI)	Date	Study Monitor/Work Assignment Manager (EPA)	Date
C Haile for JG		Frederick W. Kutz	7/16/84
Project Manager (MRI)	Date	Project Officer (EPA)	Date

APPENDIX II

DERMAL WASHING EFFICIENCY STUDIES
INDIVIDUAL ANIMAL DATA

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Table II-1. Urinary and Fecal Excretion of Radioactivity in Male Fischer 344 Rats Treated Dermally with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat): Washing Efficiency Study[a]

		Percent of dose							
		Soap and water wash				Acetone and water wash			
Excretum	Time after dosing (h)	No. 16	No. 17	No. 18	Mean ± S.E.	No. 19	No. 20	No. 21	Mean ± S.E.
Urine	6	0.104	0.395	0.193	0.231 ± 0.086	0.557	0.780	0.604	0.647 ± 0.068
	12	0.220	0.611	0.410	0.414 ± 0.113	1.601	1.356	4.184	2.380 ± 0.905
	24	0.163	0.278	0.281	0.241 ± 0.039	0.911	0.787	2.444	1.381 ± 0.533
	48	0.118	0.152	0.167	0.146 ± 0.014	0.731	0.562	0.944	0.746 ± 0.111
	72	0.050	0.093	0.104	0.082 ± 0.016	0.203	0.339	0.566	0.369 ± 0.106
	96	0.031	0.128	0.123	0.094 ± 0.032	0.191	0.387	0.508	0.362 ± 0.092
Feces	6	0.004	0.010	0.004	0.006 ± 0.002	0.004	0.006	0.007	0.006 ± 0.001
	12	0.020	0.105	0.025	0.050 ± 0.028	0.021	0.134	0.128	0.094 ± 0.037
	24	0.045	0.072	0.071	0.063 ± 0.009	0.304	0.079	0.570	0.318 ± 0.142
	48	0.109	0.151	0.162	0.141 ± 0.016	0.484	0.537	0.688	0.570 ± 0.061
	72	0.053	0.050	0.042	0.048 ± 0.003	0.179	0.272	0.660	0.370 ± 0.147
	96	0.039	0.045	0.028	0.037 ± 0.005	0.092	0.492	0.750	0.445 ± 0.191
Total	6	0.108	0.405	0.197	0.237 ± 0.088	0.561	0.786	0.611	0.653 ± 0.068
	12	0.240	0.716	0.435	0.464 ± 0.138	1.622	1.490	4.312	2.475 ± 0.919
	24	0.208	0.350	0.352	0.303 ± 0.048	1.215	0.866	3.014	1.698 ± 0.666
	48	0.227	0.303	0.329	0.286 ± 0.031	1.215	1.099	1.632	1.315 ± 0.162
	72	0.103	0.143	0.146	0.131 ± 0.014	0.382	0.611	1.226	0.740 ± 0.252
	96	0.070	0.173	0.151	0.131 ± 0.031	0.283	0.879	1.258	0.807 ± 0.284
		Cumulative percent of dose							
Urine	6	0.104	0.395	0.193	0.231 ± 0.086	0.557	0.780	0.604	0.647 ± 0.068
	12	0.324	1.006	0.603	0.644 ± 0.198	2.158	2.136	4.788	3.027 ± 0.880
	24	0.487	1.284	0.884	0.885 ± 0.230	3.069	2.923	7.232	4.408 ± 1.413
	48	0.605	1.436	1.051	1.031 ± 0.240	3.800	3.485	8.176	5.154 ± 1.514
	72	0.655	1.529	1.155	1.113 ± 0.253	4.003	3.824	8.742	5.523 ± 1.610
	96	0.686	1.657	1.278	1.207 ± 0.283	4.194	4.211	9.250	5.885 ± 1.683
Feces	6	0.004	0.010	0.004	0.006 ± 0.002	0.004	0.006	0.007	0.006 ± 0.001
	12	0.024	0.115	0.029	0.056 ± 0.030	0.025	0.140	0.135	0.100 ± 0.038
	24	0.069	0.187	0.100	0.119 ± 0.035	0.329	0.219	0.705	0.418 ± 0.147
	48	0.178	0.338	0.262	0.259 ± 0.046	0.813	0.756	1.393	0.987 ± 0.203
	72	0.231	0.388	0.304	0.308 ± 0.045	0.992	1.028	2.053	1.358 ± 0.348
	96	0.270	0.433	0.332	0.345 ± 0.048	1.084	1.520	2.803	1.802 ± 0.516
Total	6	0.108	0.405	0.197	0.237 ± 0.088	0.565	0.786	0.611	0.653 ± 0.068
	12	0.348	1.121	0.632	0.700 ± 0.226	2.183	2.276	4.923	3.127 ± 0.898
	24	0.556	1.471	0.984	1.004 ± 0.264	3.398	3.142	7.937	4.826 ± 1.557
	48	0.783	1.774	1.313	1.290 ± 0.286	4.613	4.241	9.569	6.141 ± 1.717
	72	0.886	1.917	1.459	1.421 ± 0.298	4.995	4.852	10.795	6.881 ± 1.958
	96	0.956	2.090	1.610	1.552 ± 0.329	5.278	5.731	12.053	7.687 ± 2.187
Dose wash		94.050	94.500	85.425	91.325 ± 2.953	97.425	78.388	79.463	85.092 ± 6.174
Application area		3.490	3.948	3.961	3.800 ± 0.155	10.703	14.639	12.801	12.714 ± 1.137
Recovery		98.496	100.538	90.996	96.677 ± 2.901	113.406	98.758	104.317	105.494 ± 4.269

Table II-2. Urinary and Fecal Excretion of Radioactivity in Male Hartley Guinea Pigs Treated Dermally with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig): Washing Efficiency Study

Excretum	Time after dosing (h)	Percent of dose							
		Soap and water wash				Acetone and water wash			
		No. 1	No. 2	No. 3	Mean ± S.E.	No. 4	No. 5	No. 6	Mean ± S.E.
Urine	6	0.013	0.026	0.075	0.038 ± 0.019	0.074	0.281	0.067	0.141 ± 0.070
	12	0.026	0.102	0.058	0.062 ± 0.022	0.081	0.508	0.055	0.215 ± 0.147
	24	0.040	0.124	0.139	0.101 ± 0.031	0.116	0.500	0.391	0.336 ± 0.114
	48	0.063	0.079	0.080	0.074 ± 0.006	0.341	0.461	0.315	0.372 ± 0.045
	72	0.028	0.034	0.054	0.039 ± 0.008	0.223	0.275	0.079	0.192 ± 0.059
	96	0.005	0.029	0.023	0.019 ± 0.007	0.083	0.144	0.088	0.105 ± 0.020
Feces	6	0.001	0.005	0.001	0.002 ± 0.001	0.002	-[a]	0.000	0.001
	12	0.000	0.013	0.008	0.007 ± 0.002	0.018	0.212	0.006	0.079 ± 0.067
	24	0.039	0.183	0.159	0.127 ± 0.045	0.137	0.277	0.214	0.209 ± 0.040
	48	0.055	0.151	0.084	0.097 ± 0.028	0.013	0.227	0.319	0.186 ± 0.091
	72	0.034	0.055	0.039	0.043 ± 0.006	0.111	0.198	0.256	0.188 ± 0.042
	96	0.024	0.036	0.032	0.031 ± 0.004	0.078	0.100	0.112	0.097 ± 0.010
Total	6	0.014	0.031	0.076	0.040 ± 0.018	0.076	0.281	0.067	0.141 ± 0.070
	12	0.026	0.115	0.066	0.069 ± 0.026	0.099	0.720	0.061	0.293 ± 0.214
	24	0.079	0.307	0.298	0.228 ± 0.075	0.253	0.777	0.605	0.545 ± 0.154
	48	0.118	0.230	0.164	0.171 ± 0.033	0.354	0.688	0.634	0.559 ± 0.104
	72	0.062	0.089	0.093	0.081 ± 0.010	0.334	0.473	0.335	0.381 ± 0.046
	96	0.029	0.065	0.055	0.050 ± 0.011	0.161	0.244	0.200	0.202 ± 0.024
		Cumulative percent of dose							
Urine	6	0.013	0.026	0.075	0.038 ± 0.019	0.074	0.281	0.067	0.141 ± 0.070
	12	0.039	0.128	0.133	0.100 ± 0.031	0.155	0.789	0.122	0.355 ± 0.217
	24	0.079	0.252	0.272	0.201 ± 0.061	0.271	1.289	0.513	0.691 ± 0.307
	48	0.142	0.331	0.352	0.275 ± 0.067	0.612	1.750	0.828	1.063 ± 0.349
	72	0.170	0.365	0.406	0.314 ± 0.073	0.835	2.025	0.907	1.256 ± 0.385
	96	0.175	0.394	0.429	0.333 ± 0.079	0.918	2.169	0.995	1.361 ± 0.405
Feces	6	0.001	0.005	0.001	0.002 ± 0.001	0.002	-[a]	0.000	0.001
	12	0.001	0.018	0.009	0.009 ± 0.005	0.020	0.212	0.006	0.079 ± 0.066
	24	0.040	0.201	0.168	0.136 ± 0.049	0.157	0.489	0.220	0.289 ± 0.102
	48	0.095	0.352	0.252	0.233 ± 0.075	0.170	0.716	0.539	0.475 ± 0.161
	72	0.129	0.407	0.291	0.276 ± 0.081	0.281	0.914	0.795	0.663 ± 0.194
	96	0.153	0.443	0.323	0.306 ± 0.084	0.359	1.014	0.907	0.760 ± 0.203
Total	6	0.014	0.031	0.076	0.040 ± 0.018	0.076	0.281	0.067	0.141 ± 0.070
	12	0.040	0.146	0.142	0.109 ± 0.035	0.175	1.001	0.128	0.435 ± 0.283
	24	0.119	0.453	0.440	0.337 ± 0.109	0.428	1.778	0.733	0.980 ± 0.409
	48	0.237	0.683	0.604	0.508 ± 0.137	0.782	2.466	1.367	1.538 ± 0.494
	72	0.299	0.772	0.697	0.589 ± 0.147	1.116	2.939	1.702	1.919 ± 0.537
	96	0.328	0.837	0.752	0.639 ± 0.157	1.277	3.183	1.902	2.121 ± 0.561
Dose wash		86.540	78.120	88.910	84.523 ± 3.274	81.510	67.935	78.670	76.038 ± 4.134
Application area		2.741	4.665	4.083	3.830 ± 0.570	4.652	6.837	6.250	5.913 ± 0.653
Recovery		89.609	83.622	93.745	88.992 ± 2.938	87.439	77.955	86.822	84.072 ± 3.064

[a] No feces collected during this time period.

Table II-3. Urinary and Fecal Excretion of Radioactivity in Male Rhesus Monkeys Treated Dermally with ^{14}C-Labeled 4,4'-MDA (10 mg/Monkey): Washing Efficiency Study

		Percent of dose					
		Soap and water wash			Acetone and water wash		
Excretum	Time after dosing (h)	No. 529	No. 604	Average	No. 554	No. 619	Average
Urine	6	0.506	0.054	0.280	0.272	0.394	0.333
	12	0.549	0.288	0.419	0.503	0.872	0.688
	24	2.576	0.193	1.385	0.914	2.544	1.729
	48	2.209	1.095	1.652	3.513	7.204	5.359
	72	1.530	1.453	1.492	2.281	3.329	2.805
	96	1.008	0.365	0.687	1.094	1.979	1.537
	120	0.650	0.407	0.529	0.502	1.108	0.805
	144	0.509	0.290	0.400	0.471	1.046	0.759
	168	0.445	0.364	0.405	0.355	0.564	0.460
Feces	6	0.002	0.001	0.002	0.003	0.000	0.002
	12	0.003	0.011	0.007	0.002	0.003	0.003
	24	0.017	0.018	0.018	0.022	0.099	0.061
	48	0.494	0.017	0.256	0.210	0.640	0.425
	72	0.538	0.175	0.357	0.408	0.519	0.464
	96	0.397	0.170	0.284	0.299	0.294	0.297
	120	0.260	0.184	0.222	0.170	0.273	0.222
	144	0.210	0.110	0.160	0.081	0.164	0.123
	168	0.251	0.120	0.186	0.141	0.184	0.163
		Cumulative percent of dose					
Urine	6	0.506	0.054	0.280	0.272	0.394	0.333
	12	1.055	0.342	0.699	0.775	1.266	1.021
	24	3.631	0.535	2.083	1.689	3.810	2.750
	48	5.840	1.630	3.735	5.202	11.014	8.108
	72	7.370	3.083	5.227	7.483	14.343	10.913
	96	8.378	3.448	5.913	8.577	16.322	12.450
	120	9.028	3.855	6.442	9.079	17.430	13.255
	144	9.537	4.145	6.841	9.550	18.476	14.013
	168	9.982	4.509	7.246	9.905	19.040	14.473
Feces	6	0.002	0.001	0.002	0.003	0.000	0.002
	12	0.005	0.012	0.009	0.005	0.003	0.004
	24	0.022	0.030	0.026	0.027	0.102	0.065
	48	0.516	0.047	0.282	0.237	0.742	0.490
	72	1.054	0.222	0.638	0.645	1.261	0.953
	96	1.451	0.392	0.922	0.944	1.555	1.250
	120	1.711	0.576	1.144	1.114	1.828	1.471
	144	1.921	0.686	1.304	1.195	1.992	1.594
	168	2.172	0.806	1.490	1.336	2.176	1.756
Dose wash		54.294	71.663	62.979	59.741	46.266	53.004
Recovery		66.448	76.978	71.713	70.982	67.482	69.232

APPENDIX III

RAT STUDIES
INDIVIDUAL ANIMAL DATA

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Table III-1. Urinary and Fecal Excretion of Radioactivity in Male Fischer 344 Rats Treated Intravenously with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)

		Percent of dose							
Excretum	Time after dosing (h)	No. 58	No. 59	No. 60	Mean ± S.E.	No. 49	No. 50	No. 51	Mean ± S.E.
Urine	6	47.128	34.264	32.322	37.905 ± 4.646	38.282	35.019	35.432	36.244 ± 1.026
	12	16.678	20.547	24.772	20.666 ± 2.337	16.554	19.220	20.788	18.854 ± 1.236
	24	6.689	9.818	9.822	8.776 ± 1.044	9.142	9.539	7.529	8.737 ± 0.615
	48					1.801	1.579	1.932	1.771 ± 0.103
	72					1.165	0.903	0.639	0.902 ± 0.152
	96					0.459	0.463	0.423	0.448 ± 0.013
Feces	6	0.263	0.164	0.168	0.198 ± 0.032	3.775	0.536	5.007	3.106 ± 1.333
	12	9.923	0.387	3.507	4.606 ± 2.807	13.073	14.215	12.626	13.305 ± 0.473
	24	13.872	21.867	15.222	16.987 ± 2.471	9.185	15.356	9.139	11.227 ± 2.065
	48					2.175	1.934	1.944	2.018 ± 0.079
	72					0.708	0.409	0.415	0.511 ± 0.099
	96					0.833	0.297	0.360	0.497 ± 0.169
Total	6	47.391	34.428	32.490	38.103 ± 4.678	42.057	35.555	40.439	39.350 ± 1.954
	12	26.601	20.934	28.279	25.271 ± 2.222	29.627	33.435	33.414	32.159 ± 1.266
	24	20.561	31.685	25.044	25.763 ± 3.231	18.327	24.895	16.668	19.963 ± 2.512
	48					3.976	3.513	3.876	3.788 ± 0.141
	72					1.873	1.312	1.054	1.413 ± 0.242
	96					1.292	0.760	0.783	0.945 ± 0.174
		Cumulative percent of dose							
Urine	6	47.128	34.264	32.322	37.905 ± 4.646	38.282	35.019	35.432	36.244 ± 1.026
	12	63.806	54.811	57.094	58.570 ± 2.700	54.836	54.239	56.220	55.098 ± 0.587
	24	70.495	64.629	66.916	67.347 ± 1.707	63.978	63.778	63.749	63.835 ± 0.072
	48					65.779	65.357	65.681	65.606 ± 0.128
	72					66.944	66.260	66.320	66.508 ± 0.219
	96					67.403	66.723	66.743	66.956 ± 0.223
Feces	6	0.263	0.164	0.168	0.198 ± 0.032	3.775	0.536	5.007	3.106 ± 1.333
	12	10.186	0.551	3.675	4.804 ± 2.838	16.848	14.751	17.633	16.411 ± 0.860
	24	24.058	22.418	18.897	21.791 ± 1.522	26.033	30.107	26.772	27.637 ± 1.253
	48					28.208	32.041	28.716	29.655 ± 1.202
	72					28.916	32.450	29.131	30.166 ± 1.144
	96					29.749	32.747	29.491	30.662 ± 1.045
Total	6	47.391	34.428	32.490	38.103 ± 4.678	42.057	35.555	40.439	39.350 ± 1.954
	12	73.992	55.362	60.769	63.374 ± 5.534	71.684	68.990	73.853	71.509 ± 1.407
	24	94.553	87.047	85.813	89.138 ± 2.731	90.011	93.885	90.521	91.472 ± 1.215
	48					93.987	97.398	94.397	95.261 ± 1.075
	72					95.860	98.710	95.451	96.674 ± 1.025
	96					97.152	99.470	96.234	97.619 ± 0.963

Table III-2. Radioactivity in Blood and Tissue at 6, 24, and 96 h Following Intravenous Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)

Tissue	µg equivalents/g or mL											
	6 h				24 h				96 h			
	No. 55	No. 56	No. 57	Mean ± S.E.	No. 58	No. 59	No. 60	Mean ± S.E.	No. 49	No. 50	No. 51	Mean ± S.E.
Blood	1.077	0.916	0.911	0.968 ± 0.055	0.133	0.172	0.155	0.153 ± 0.011	0.064	0.059	0.061	0.061 ± 0.001
Plasma	1.370	1.039	1.005	1.138 ± 0.116	0.143	0.186	0.159	0.163 ± 0.013	0.051	0.041	0.045	0.046 ± 0.003
RBCs	0.536	0.411	0.476	0.474 ± 0.036	0.103	0.136	0.135	0.125 ± 0.011	0.065	0.062	0.063	0.063 ± 0.001
Liver	4.738	4.578	5.268	4.861 ± 0.209	1.295	1.784	1.072	1.384 ± 0.210	0.304	0.285	0.335	0.308 ± 0.015
Kidneys	1.082	0.871	0.922	0.958 ± 0.064	0.080	0.097	0.105	0.094 ± 0.007	0.037	0.063	0.055	0.052 ± 0.008
Lungs	1.408	1.061	0.984	1.151 ± 0.130	1.159	0.458	0.413	0.677 ± 0.242	0.068	0.070	0.074	0.071 ± 0.002
Brain	0.143	0.140	0.149	0.144 ± 0.003	0.007	0.008	0.009	0.008 ± 0.001	0.004	0.004	0.004	0.004 ± 0.000
Spleen	1.000	0.795	0.860	0.885 ± 0.060	0.410	0.375	0.453	0.413 ± 0.023	0.205	0.214	0.193	0.204 ± 0.006
Testes	0.839	0.527	0.514	0.627 ± 0.106	0.012	0.018	0.017	0.016 ± 0.002	0.005	0.005	0.005	0.005 ± 0.000
Adrenals	0.585	1.005	0.810	0.800 ± 0.121	0.112	0.134	0.196	0.147 ± 0.025	0.088	0.110	0.112	0.103 ± 0.008
Bladder	0.054	0.022	0.086	0.054 ± 0.018	0.003	0.001	0.007	0.004 ± 0.002	0.005	0.004	0.004	0.004 ± 0.000
Muscle	0.156	0.157	0.187	0.167 ± 0.010	0.008	0.013	0.014	0.012 ± 0.002	0.010	0.013	0.009	0.011 ± 0.001
Fat	0.023	0.027	0.022	0.024 ± 0.002	0.007	0.011	0.009	0.009 ± 0.001	0.006	0.005	0.006	0.006 ± 0.000
GI tract	5.961	5.920	5.103	5.661 ± 0.279	0.698	0.590	0.620	0.636 ± 0.032	0.013	0.018	0.015	0.015 ± 0.001
Skin	0.452	0.491	0.501	0.481 ± 0.015	0.028	0.037	0.048	0.038 ± 0.006	0.019	0.018	0.022	0.020 ± 0.001

Table III-2 (continued)

Tissue/ excretum	Percent of administered dose											
	6 h				24 h				96 h			
	No. 55	No. 56	No. 57	Mean ± S.E.	No. 58	No. 59	No. 60	Mean ± S.E.	No. 49	No. 50	No. 51	Mean ± S.E.
Blood[a]	2.978	2.645	2.774	2.799 ± 0.097	0.396	0.480	0.455	0.444 ± 0.025	0.232	0.208	0.214	0.218 ± 0.007
Plasma[a,b]	2.273	1.800	1.836	1.970 ± 0.152	0.255	0.312	0.281	0.283 ± 0.016	0.111	0.088	0.096	0.098 ± 0.007
RBCs[a,b]	0.593	0.474	0.580	0.549 ± 0.038	0.123	0.152	0.159	0.145 ± 0.011	0.094	0.088	0.089	0.090 ± 0.002
Liver	8.445	9.381	10.577	9.468 ± 0.617	3.483	4.437	2.706	3.542 ± 0.501	0.887	0.860	0.834	0.860 ± 0.015
Kidneys	0.468	0.430	0.537	0.478 ± 0.031	0.044	0.051	0.058	0.051 ± 0.004	0.025	0.039	0.033	0.032 ± 0.004
Lungs	0.329	0.237	0.200	0.255 ± 0.038	0.381	0.167	0.156	0.235 ± 0.073	0.016	0.015	0.015	0.015 ± 0.000
Brain	0.080	0.074	0.082	0.079 ± 0.002	0.006	0.006	0.006	0.006 ± 0.000	0.002	0.002	0.002	0.002 ± 0.000
Spleen	0.095	0.092	0.092	0.093 ± 0.001	0.054	0.051	0.060	0.055 ± 0.003	0.025	0.025	0.023	0.024 ± 0.001
Testes	0.325	0.213	0.248	0.262 ± 0.033	0.006	0.008	0.008	0.007 ± 0.001	0.003	0.003	0.003	0.003 ± 0.000
Adrenals	0.004	0.010	0.006	0.007 ± 0.002	0.001	0.002	0.002	0.002 ± 0.000	0.001	0.001	0.001	0.001 ± 0.000
Bladder	0.001	0.000	0.001	0.001 ± 0.000	0.000	0.000	0.000	0.000 ± 0.000	0.000	0.001	0.000	0.000 ± 0.000
Muscle[a]	2.457	2.591	3.254	2.767 ± 0.246	0.136	0.200	0.227	0.188 ± 0.027	0.207	0.254	0.172	0.211 ± 0.024
Urine	53.856	60.055	50.043	54.651 ± 2.918	70.495	64.629	66.916	67.347 ± 1.707	67.403	66.723	66.743	66.956 ± 0.223
Feces	0.770	0.015	0.056	0.280 ± 0.245	24.058	22.418	18.897	21.791 ± 1.522	29.749	32.747	29.491	30.662 ± 1.045
GI tract	22.279	27.331	22.204	23.938 ± 1.697	4.678	3.777	3.975	4.143 ± 0.273	0.109	0.140	0.078	0.109 ± 0.018
Skin[a]	2.854	3.238	3.487	3.193 ± 0.184	0.191	0.237	0.319	0.249 ± 0.037	0.153	0.146	0.178	0.159 ± 0.010
Recovery[b]	94.941	106.312	93.561	98.271 ± 4.040	103.929	96.463	93.785	98.059 ± 3.035	98.812	101.164	97.787	99.254 ± 0.999

[a] Calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[b] Individual blood components and fat are not included in recovery estimates.

Table III-3. Recovery of Radioactivity in Blood, Tissue, and Excreta at 6, 24, or 96 h Following Intravenous Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)

Tissue/ excretum	Percent of dose											
	6 h				24 h				96 h			
	No. 55	No. 56	No. 57	Mean ± S.E.	No. 58	No. 59	No. 60	Mean ± S.E.	No. 49	No. 50	No. 51	Mean ± S.E.
Blood	2.978	2.645	2.774	2.799 ± 0.097	0.396	0.480	0.455	0.444 ± 0.025	0.232	0.208	0.214	0.218 ± 0.007
Tissue	15.058	16.266	18.484	16.603 ± 1.003	4.302	5.159	3.542	4.334 ± 0.467	1.319	1.346	1.261	1.308 ± 0.025
GI tract	22.279	27.331	22.204	23.938 ± 1.697	4.678	3.777	3.975	4.143 ± 0.273	0.109	0.140	0.078	0.109 ± 0.018
Urine	53.856	60.055	50.043	54.651 ± 2.918	70.495	64.629	66.916	67.347 ± 1.707	67.403	66.723	66.743	66.956 ± 0.223
Feces	0.770	0.015	0.056	0.280 ± 0.245	24.058	22.418	18.897	21.791 ± 1.522	29.749	32.747	29.491	30.662 ± 1.045
Recovery	94.941	106.312	93.561	98.271 ± 4.040	103.929	96.463	93.785	98.059 ± 3.035	98.812	101.164	97.787	99.254 ± 0.999

Table III-4. Urinary and Fecal Excretion of Radioactivity in Male Fischer 344 Rats Treated Dermally with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)

Excretum	Time after dosing (h)	24-h sacrifice				96-h sacrifice			
		No. 34	No. 35	No. 36	Mean ± S.E.	No. 40	No. 41	No. 42	Mean ± S.E.
		Percent of dose							
Urine	6	2.706	2.498	1.415	2.206 ± 0.400	1.554	2.840	0.145	1.513 ± 0.778
	12	5.265	7.390	1.764	4.806 ± 1.640	8.135	5.555	11.071	8.254 ± 1.593
	24	16.071	13.473	9.507	13.017 ± 1.909	14.201	13.164	13.686	13.684 ± 0.299
	48					11.326	10.846	15.791	12.654 ± 1.574
	72					5.005	3.146	5.594	4.582 ± 0.738
	96					3.042	1.850	2.175	2.356 ± 0.356
Feces	6	0.018	0.051	0.024	0.031 ± 0.010	0.002	-[a]	0.027	0.015
	12	0.439	0.776	-[a]	0.608	1.081	0.419	0.777	0.759 ± 0.191
	24	1.678	1.888	2.048	1.871 ± 0.107	3.171	3.007	1.888	2.689 ± 0.403
	48					3.527	3.094	4.909	3.843 ± 0.547
	72					1.381	2.261	1.249	1.630 ± 0.318
	96					1.287	0.869	0.930	1.029 ± 0.130
Total	6	2.724	2.549	1.439	2.237 ± 0.402	1.556	2.840	0.172	1.523 ± 0.770
	12	5.704	8.166	1.764	5.211 ± 1.864	9.216	5.974	11.848	9.013 ± 1.699
	24	17.749	15.361	11.555	14.888 ± 1.804	17.372	16.171	15.574	16.372 ± 0.529
	48					14.853	13.940	20.700	16.498 ± 2.118
	72					6.386	5.407	6.843	6.212 ± 0.424
	96					4.329	2.719	3.105	3.384 ± 0.485
		Cumulative percent of dose							
Urine	6	2.706	2.498	1.415	2.206 ± 0.400	1.554	2.840	0.145	1.513 ± 0.778
	12	7.971	9.888	3.179	7.013 ± 1.995	9.689	8.395	11.216	9.767 ± 0.815
	24	24.042	23.361	12.686	20.030 ± 3.677	23.890	21.559	24.902	23.450 ± 0.990
	48					35.216	32.405	40.693	36.105 ± 2.433
	72					40.221	35.551	46.287	40.686 ± 3.108
	96					43.263	37.401	48.462	43.042 ± 3.195
Feces	6	0.018	0.051	0.024	0.031 ± 0.010	0.002	-[a]	0.027	0.015
	12	0.457	0.827	0.024	0.436 ± 0.232	1.083	0.419	0.804	0.769 ± 0.192
	24	2.135	2.715	2.072	2.307 ± 0.205	4.254	3.426	2.692	3.457 ± 0.451
	48					7.781	6.520	7.601	7.301 ± 0.394
	72					9.162	8.781	8.850	8.931 ± 0.117
	96					10.449	9.650	9.780	9.960 ± 0.248
Total	6	2.724	2.549	1.439	2.237 ± 0.402	1.556	2.840	0.172	1.523 ± 0.770
	12	8.428	10.715	3.203	7.449 ± 2.223	10.772	8.814	12.020	10.535 ± 0.933
	24	26.177	26.076	14.758	22.337 ± 3.790	28.144	24.985	27.594	26.908 ± 0.974
	48					42.997	38.925	48.294	43.405 ± 2.712
	72					49.383	44.332	55.137	49.617 ± 3.121
	96					53.712	47.051	58.242	53.002 ± 3.250

[a] No feces collected during this time period.

Table III-5. Radioactivity in Blood and Tissue at 6, 24, and 96 h Following Dermal Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)

Tissue	µg equivalents/g or mL											
	6 h				24 h				96 h			
	No. 31	No. 32	No. 33	Mean ± S.E.	No. 34	No. 35	No. 36	Mean ± S.E.	No. 40	No. 41	No. 42	Mean ± S.E.
Blood	0.154	0.289	0.200	0.214 ± 0.040	0.097	0.096	0.047	0.080 ± 0.017	0.032	0.027	0.031	0.030 ± 0.002
Plasma	0.179	0.343	0.227	0.250 ± 0.049	0.103	0.103	0.048	0.085 ± 0.018	0.028	0.021	0.026	0.025 ± 0.002
RBCs	0.075	0.158	0.124	0.119 ± 0.024	0.045	0.053	0.031	0.043 ± 0.006	0.024	0.025	0.029	0.026 ± 0.002
Liver	0.626	1.067	0.874	0.856 ± 0.128	0.549	0.639	0.463	0.550 ± 0.051	0.289	0.254	0.276	0.273 ± 0.010
Kidneys	0.218	0.320	0.243	0.260 ± 0.031	0.144	0.140	0.074	0.119 ± 0.023	0.068	0.050	0.058	0.059 ± 0.005
Lungs	0.061	0.196	0.130	0.129 ± 0.039	0.042	0.056	0.021	0.040 ± 0.010	0.015	0.014	0.016	0.015 ± 0.001
Brain	0.039	0.085	0.052	0.059 ± 0.014	0.015	0.017	0.007	0.013 ± 0.003	0.003	0.002	0.003	0.003 ± 0.000
Spleen	0.075	0.132	0.096	0.101 ± 0.017	0.036	0.038	0.020	0.031 ± 0.006	0.014	0.011	0.014	0.013 ± 0.001
Testes	0.092	0.163	0.131	0.129 ± 0.021	0.046	0.054	0.022	0.041 ± 0.010	0.008	0.005	0.007	0.007 ± 0.001
Adrenals	0.181	0.358	0.231	0.257 ± 0.053	0.122	0.098	0.064	0.095 ± 0.017	0.051	0.041	0.060	0.051 ± 0.005
Bladder	0.002	0.005	0.036	0.014 ± 0.011	-^{c}	0.004	0.001	0.003	0.003	0.002	0.032	0.012 ± 0.010
Muscle	0.043	0.074	0.057	0.058 ± 0.009	0.022	0.028	0.013	0.021 ± 0.004	0.005	0.006	0.004	0.005 ± 0.001
Fat	0.015	0.013	0.015	0.014 ± 0.001	0.004	0.031	0.003	0.013 ± 0.009	0.002	0.002	0.002	0.002 ± 0.000
GI tract	0.326	1.546	0.697	0.856 ± 0.361	0.955	0.943	0.317	0.738 ± 0.211	0.119	0.084	0.071	0.091 ± 0.014
Nontreated skin	0.108	0.193	0.154	0.152 ± 0.025	0.061	0.062	0.028	0.050 ± 0.011	0.028	0.025	0.020	0.024 ± 0.002

Table III-5 (continued)

Tissue/ excretum	Percent of administered dose											
	6 h				24 h				96 h			
	No. 31	No. 32	No. 33	Mean ± S.E.	No. 34	No. 35	No. 36	Mean ± S.E.	No. 40	No. 41	No. 42	Mean ± S.E.
Blood[a]	0.537	1.007	0.763	0.769 ± 0.136	0.355	0.345	0.165	0.288 ± 0.062	0.116	0.097	0.107	0.107 ± 0.005
Plasma[a,b]	0.373	0.717	0.520	0.537 ± 0.100	0.226	0.221	0.100	0.182 ± 0.041	0.061	0.045	0.054	0.053 ± 0.005
RBCs[a,b]	0.105	0.220	0.188	0.171 ± 0.034	0.066	0.076	0.043	0.062 ± 0.010	0.035	0.036	0.040	0.037 ± 0.002
Liver	1.364	2.398	2.123	1.962 ± 0.309	1.300	1.331	0.953	1.195 ± 0.121	0.580	0.486	0.559	0.542 ± 0.028
Kidneys	0.125	0.173	0.146	0.148 ± 0.014	0.085	0.082	0.043	0.070 ± 0.014	0.028	0.022	0.025	0.025 ± 0.002
Lungs	0.015	0.051	0.033	0.033 ± 0.010	0.010	0.014	0.005	0.010 ± 0.003	0.003	0.003	0.003	0.003 ± 0.000
Brain	0.021	0.049	0.025	0.032 ± 0.009	0.009	0.009	0.004	0.007 ± 0.002	0.002	0.001	0.002	0.002 ± 0.000
Spleen	0.010	0.017	0.014	0.014 ± 0.002	0.004	0.005	0.002	0.004 ± 0.001	0.002	0.001	0.002	0.002 ± 0.000
Testes	0.051	0.089	0.082	0.074 ± 0.012	0.027	0.033	0.012	0.024 ± 0.006	0.005	0.003	0.004	0.004 ± 0.001
Adrenals	0.002	0.004	0.003	0.003 ± 0.001	0.002	0.002	0.001	0.002 ± 0.000	0.001	0.001	0.001	0.001 ± 0.000
Bladder	0.000	0.000	0.001	0.000 ± 0.000	-[c]	0.000	0.000	0.000	0.000	0.000	0.001	0.000 ± 0.000
Muscle[a]	0.856	1.473	1.243	1.191 ± 0.180	0.462	0.564	0.260	0.429 ± 0.089	0.095	0.113	0.080	0.096 ± 0.010
GI tract	1.397	6.741	3.246	3.795 ± 1.567	4.028	3.860	1.043	2.977 ± 0.968	0.629	0.409	0.414	0.484 ± 0.073
Nontreated skin[a]	0.856	1.533	1.343	1.244 ± 0.202	0.513	0.509	0.220	0.414 ± 0.097	0.236	0.205	0.160	0.200 ± 0.022
Urine	2.652	2.748	2.242	2.547 ± 0.155	24.042	23.361	12.686	20.030 ± 3.677	43.263	37.401	48.462	43.042 ± 3.195
Feces	0.050	0.028	0.035	0.038 ± 0.006	2.135	2.715	2.072	2.307 ± 0.205	10.449	9.650	9.780	9.960 ± 0.248
Dose wash	75.675	57.275	53.275	62.075 ± 6.897	43.050	49.525	63.675	52.083 ± 6.090	21.775	30.375	21.850	24.667 ± 2.854
Application area	27.900	28.913	34.950	30.588 ± 2.201	31.510	27.896	18.126	25.844 ± 3.998	26.242	24.244	26.150	25.545 ± 0.651
Recovery[b]	111.511	102.499	99.524	104.511 ± 3.604	107.532	110.251	99.267	105.683 ± 3.303	103.426	103.011	107.600	104.679 ± 1.465

[a] Calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[b] Individual blood components and fat are not included in recovery estimates.

[c] Sample lost.

Table III-6. Recovery of Radioactivity in Blood, Tissue, and Excreta at 6, 24, or 96 h Following Dermal Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat)

Tissue/ excretum	Percent of dose											
	6 h				24 h				96 h			
	No. 31	No. 32	No. 33	Mean ± S.E.	No. 34	No. 35	No. 36	Mean ± S.E.	No. 40	No. 41	No. 42	Mean ± S.E.
Blood	0.537	1.007	0.763	0.769 ± 0.136	0.355	0.345	0.165	0.288 ± 0.062	0.116	0.097	0.107	0.107 ± 0.005
Tissue	3.300	5.787	5.013	4.700 ± 0.735	2.412	2.549	1.500	2.154 ± 0.329	0.952	0.835	0.837	0.875 ± 0.039
GI tract	1.397	6.741	3.246	3.795 ± 1.567	4.028	3.860	1.043	2.977 ± 0.968	0.629	0.409	0.414	0.484 ± 0.073
Urine	2.652	2.748	2.242	2.547 ± 0.155	24.042	23.361	12.686	20.030 ± 3.677	43.263	37.401	48.462	43.042 ± 3.195
Feces	0.050	0.028	0.035	0.038 ± 0.006	2.135	2.715	2.072	2.307 ± 0.205	10.449	9.650	9.780	9.960 ± 0.248
Total absorbed	7.936	16.311	11.299	11.849 ± 2.433	32.972	32.830	17.466	27.756 ± 5.145	55.409	48.392	59.600	54.467 ± 3.270
Dose wash	75.675	57.275	53.275	62.075 ± 6.897	43.050	49.525	63.675	52.083 ± 6.090	21.775	30.375	21.850	24.667 ± 2.854
Application area	27.900	28.913	34.950	30.588 ± 2.201	31.510	27.896	18.126	25.844 ± 3.998	26.242	24.244	26.150	25.545 ± 0.651
Recovery	111.511	102.499	99.524	104.511 ± 3.604	107.532	110.251	99.267	105.683 ± 3.303	103.426	103.011	107.600	104.679 ± 1.465

Table III-7. Radioactivity in Blood, Tissue, and Excreta at 6 h Following Dermal Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat): Nonoccluded Application Area

Tissue/ excretum	µg equivalents/g or mL				Percent of dose			
	No. 28	No. 29	No. 30	Mean ± S.E.	No. 28	No. 29	No. 30	Mean ± S.E.
Blood[a]	0.192	0.096	0.153	0.147 ± 0.028	0.672	0.360	0.547	0.526 ± 0.091
Plasma[a,b]	0.176	0.110	0.173	0.153 ± 0.022	0.369	0.249	0.373	0.330 ± 0.041
RBCs[a,b]	0.200	0.065	0.078	0.114 ± 0.043	0.280	0.098	0.112	0.163 ± 0.058
Liver	0.687	0.511	0.661	0.620 ± 0.055	1.509	1.243	1.508	1.420 ± 0.089
Kidneys	0.185	0.137	0.201	0.174 ± 0.019	0.098	0.085	0.117	0.100 ± 0.009
Lungs	0.135	0.050	0.089	0.091 ± 0.025	0.031	0.014	0.020	0.022 ± 0.005
Brain	0.037	0.029	0.038	0.035 ± 0.003	0.018	0.015	0.021	0.018 ± 0.002
Spleen	0.056	0.056	0.058	0.057 ± 0.001	0.007	0.007	0.007	0.007 ± 0.000
Testes	0.104	0.062	0.095	0.087 ± 0.013	0.054	0.038	0.055	0.049 ± 0.006
Adrenals	0.173	0.116	0.161	0.150 ± 0.017	0.002	0.002	0.002	0.002 ± 0.000
Bladder	0.019	0.001	0.005	0.008 ± 0.005	0.001	0.000	0.000	0.000 ± 0.000
Muscle[a]	0.046	0.043	0.048	0.046 ± 0.001	0.910	0.914	0.985	0.936 ± 0.024
Fat[b]	0.008	0.008	0.012	0.009 ± 0.001	-	-	-	-
GI tract	0.310	0.214	0.334	0.286 ± 0.037	1.410	0.880	1.419	1.236 ± 0.178
Nontreated skin[a]	0.096	0.063	0.100	0.086 ± 0.012	0.764	0.542	0.821	0.709 ± 0.085
Urine	-	-	-	-	2.127	1.342	2.369	1.946 ± 0.310
Feces	-	-	-	-	0.031	0.023	0.027	0.027 ± 0.002
Dose wash	-	-	-	-	61.775	86.150	62.700	70.208 ± 7.975
Application area	-	-	-	-	30.050	15.800	36.750	27.533 ± 6.177
Recovery[b]	-	-	-	-	99.459	107.415	107.348	104.741 ± 2.641

[a] Percent of dose calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively.
Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[b] Individual blood components and fat are not included in recovery estimates.

Table III-8. Recovery of Radioactivity in Blood, Tissue, and Excreta at 6 h Following Dermal Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat): Nonoccluded Application Area

Tissue/ excretum	Percent of dose			
	No. 28	No. 29	No. 30	Mean ± S.E.
Blood	0.672	0.360	0.547	0.526 ± 0.091
Tissue	3.394	2.860	3.536	3.263 ± 0.206
GI tract	1.410	0.880	1.419	1.236 ± 0.178
Urine	2.127	1.342	2.369	1.946 ± 0.310
Feces	0.031	0.023	0.027	0.027 ± 0.002
Total absorbed	7.634	5.465	7.898	6.999 ± 0.771
Dose wash	61.775	86.150	62.700	70.208 ± 7.975
Application area	30.050	15.800	36.750	27.533 ± 6.177
Recovery	99.459	107.415	107.348	104.741 ± 2.641

Table III-9. Urinary and Fecal Excretion of Radioactivity Following Dermal Application of ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat) to Male Fischer 344 Rats: Application Area Washed at 24 h

Excretum	Time after dosing (h)	Percent of dose				Cumulative percent of dose			
		No. 37	No. 38	No. 39	Mean ± S.E.	No. 37	No. 38	No. 39	Mean ± S.E.
Urine	6	2.658	1.984	2.413	2.352 ± 0.197	2.658	1.984	2.413	2.352 ± 0.197
	12	6.761	8.128	7.005	7.298 ± 0.421	9.419	10.112	9.418	9.650 ± 0.231
	24	15.008	18.206	14.443	15.886 ± 1.172	24.427	28.318	23.861	25.535 ± 1.401
	48	5.838	7.392	5.458	6.229 ± 0.592	30.265	35.710	29.319	31.765 ± 1.991
	72	0.535	0.849	0.840	0.741 ± 0.103	30.800	36.559	30.159	32.506 ± 2.035
	96	0.549	0.557	1.058	0.721 ± 0.168	31.349	37.116	31.217	33.227 ± 1.945
Feces	6	0.046	0.008	-[a]	0.027	0.046	0.008	-[a]	0.027
	12	0.153	0.138	0.022	0.104 ± 0.041	0.199	0.146	0.022	0.122 ± 0.052
	24	4.086	4.543	4.713	4.447 ± 0.187	4.285	4.689	4.735	4.570 ± 0.143
	48	3.401	5.369	2.403	3.724 ± 0.871	7.686	10.058	7.138	8.294 ± 0.896
	72	0.485	0.780	0.521	0.595 ± 0.093	8.171	10.838	7.659	8.889 ± 0.985
	96	0.221	0.291	0.358	0.290 ± 0.040	8.392	11.129	8.017	9.179 ± 0.981
Total	6	2.704	1.992	2.413	2.370 ± 0.207	2.704	1.992	2.413	2.370 ± 0.207
	12	6.914	8.266	7.027	7.402 ± 0.433	9.618	10.258	9.440	9.772 ± 0.248
	24	19.094	22.749	19.156	20.333 ± 1.208	28.712	33.007	28.596	30.105 ± 1.451
	48	9.239	12.761	7.861	9.954 ± 1.459	37.951	45.768	36.457	40.059 ± 2.887
	72	1.020	1.629	1.361	1.337 ± 0.176	38.971	47.397	37.818	41.395 ± 3.019
	96	0.770	0.848	1.416	1.011 ± 0.204	39.741	48.245	39.234	42.407 ± 2.923

[a] No feces collected during this time period.

Table III-10. Radioactivity in Blood, Tissue, and Excreta at 96 h Following Dermal Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat): Application Area Washed at 24 h

Tissue/ excretum	µg equivalents/g or mL				Percent of dose			
	No. 37	No. 38	No. 39	Mean ± S.E.	No. 37	No. 38	No. 39	Mean ± S.E.
Blood[a]	0.018	0.023	0.017	0.019 ± 0.002	0.066	0.081	0.058	0.068 ± 0.007
Plasma[a,b]	0.011	0.013	0.013	0.012 ± 0.001	0.023	0.027	0.026	0.025 ± 0.001
RBCs[a,b]	0.021	0.027	0.020	0.023 ± 0.002	0.031	0.038	0.028	0.032 ± 0.003
Liver	0.139	0.157	0.102	0.133 ± 0.016	0.201	0.222	0.191	0.205 ± 0.009
Kidneys	0.030	0.036	0.031	0.032 ± 0.002	0.011	0.013	0.013	0.012 ± 0.001
Lungs	0.006	0.008	0.007	0.007 ± 0.001	0.001	0.002	0.002	0.002 ± 0.000
Brain	0.001	0.001	0.001	0.001 ± 0.000	0.001	0.001	0.001	0.001 ± 0.000
Spleen	0.007	0.007	0.007	0.007 ± 0.000	0.001	0.001	0.001	0.001 ± 0.000
Testes	0.002	0.002	0.003	0.002 ± 0.000	0.001	0.001	0.002	0.001 ± 0.000
Adrenals	0.000	0.024	0.003	0.009 ± 0.008	0.000	0.000	0.000	0.000 ± 0.000
Bladder	0.001	0.002	0.001	0.001 ± 0.000	0.000	0.000	0.000	0.000 ± 0.000
Muscle[a]	0.004	0.002	0.002	0.003 ± 0.001	0.074	0.041	0.040	0.052 ± 0.011
Fat[b]	0.001	0.002	0.001	0.001 ± 0.000	-	-	-	-
GI tract	0.042	0.031	0.010	0.028 ± 0.009	0.103	0.092	0.053	0.083 ± 0.015
Nontreated skin[a]	0.011	0.016	0.011	0.013 ± 0.002	0.093	0.127	0.084	0.101 ± 0.013
Urine	-	-	-	-	31.349	37.116	31.217	33.227 ± 1.945
Feces	-	-	-	-	8.392	11.129	8.017	9.179 ± 0.991
Dose wash	-	-	-	-	54.650	45.950	55.750	52.117 ± 3.100
Application area	-	-	-	-	9.830	13.477	8.694	10.667 ± 1.443
Recovery[b]	-	-	-	-	104.773	108.253	104.123	105.716 ± 1.282

[a] Percent of dose calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively.
Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[b] Individual blood components and fat are not included in recovery estimates.

Table III-11. Recovery of Radioactivity in Blood, Tissue, and Excreta at 96 h Following Dermal Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (0.4 mg/Rat): Application Area Washed at 24 h

Tissue/ excretum	Percent of dose			
	No. 37	No. 38	No. 39	Mean ± S.E.
Blood	0.066	0.081	0.058	0.068 ± 0.007
Tissue	0.383	0.408	0.334	0.375 ± 0.022
GI tract	0.103	0.092	0.053	0.083 ± 0.015
Urine	31.349	37.116	31.217	33.227 ± 1.945
Feces	8.392	11.129	8.017	9.179 ± 0.981
Total absorbed	40.293	48.826	39.679	42.933 ± 2.952
Dose wash	54.650	45.950	55.750	52.117 ± 3.100
Application area	9.830	13.477	8.694	10.667 ± 1.443
Recovery	104.773	108.253	104.123	105.716 ± 1.282

Table III-12. Urinary and Fecal Excretion of Radioactivity in Male Fischer 344 Rats Treated Dermally with ^{14}C-Labeled 4,4'-MDA (4.0 mg/Rat): Continuous 96-h Application versus Washing at 24 h

Excretum	Time after dosing (h)	Percent of dose							
		Continuous				24-h Wash			
		No. 46	No. 47	No. 48	Mean ± S.E.	No. 43	No. 44	No. 45	Mean ± S.E.
Urine	6	0.142	0.127	0.177	0.149 ± 0.015	0.068	0.083	0.081	0.077 ± 0.005
	12	0.278	0.443	0.392	0.371 ± 0.049	0.221	0.114	0.433	0.256 ± 0.094
	24	0.691	0.577	0.739	0.669 ± 0.048	0.695	0.525	0.605	0.608 ± 0.049
	48	1.533	1.466	1.169	1.389 ± 0.112	1.084	1.890	1.375	1.450 ± 0.236
	72	1.542	1.197	0.832	1.190 ± 0.205	0.302	1.245	1.478	1.008 ± 0.360
	96	1.229	1.213	0.699	1.047 ± 0.174	0.188	0.739	0.882	0.603 ± 0.212
Feces	6	0.006	0.003	0.010	0.006 ± 0.002	0.003	0.001	0.003	0.002 ± 0.001
	12	0.047	0.064	0.060	0.057 ± 0.005	0.029	0.039	0.076	0.048 ± 0.014
	24	0.155	0.181	0.187	0.174 ± 0.010	0.157	0.089	0.174	0.140 ± 0.026
	48	0.458	0.294	0.316	0.356 ± 0.051	0.297	0.394	0.419	0.370 ± 0.037
	72	0.448	0.287	0.247	0.327 ± 0.061	0.149	0.380	0.334	0.288 ± 0.071
	96	0.450	0.535	0.257	0.414 ± 0.082	0.075	0.236	0.330	0.214 ± 0.074
Total	6	0.148	0.130	0.187	0.155 ± 0.017	0.071	0.084	0.084	0.080 ± 0.004
	12	0.325	0.507	0.452	0.428 ± 0.054	0.250	0.153	0.509	0.304 ± 0.106
	24	0.846	0.758	0.926	0.843 ± 0.049	0.852	0.614	0.779	0.748 ± 0.070
	48	1.991	1.760	1.485	1.745 ± 0.146	1.381	2.284	1.794	1.820 ± 0.261
	72	1.990	1.484	1.079	1.518 ± 0.264	0.451	1.625	1.812	1.296 ± 0.426
	96	1.679	1.748	0.956	1.461 ± 0.253	0.263	0.975	1.212	0.816 ± 0.285
		Cumulative percent of dose							
Urine	6	0.142	0.127	0.177	0.149 ± 0.015	0.068	0.083	0.081	0.077 ± 0.005
	12	0.420	0.570	0.569	0.520 ± 0.050	0.289	0.197	0.514	0.333 ± 0.094
	24	1.111	1.147	1.308	1.189 ± 0.061	0.984	0.722	1.119	0.942 ± 0.117
	48	2.644	2.613	2.477	2.578 ± 0.051	2.068	2.612	2.494	2.391 ± 0.165
	72	4.186	3.810	3.309	3.768 ± 0.254	2.370	3.857	3.972	3.400 ± 0.516
	96	5.415	5.023	4.008	4.815 ± 0.419	2.558	4.596	4.854	4.003 ± 0.726
Feces	6	0.006	0.003	0.010	0.006 ± 0.002	0.003	0.001	0.003	0.002 ± 0.001
	12	0.053	0.067	0.070	0.063 ± 0.005	0.032	0.040	0.079	0.050 ± 0.015
	24	0.208	0.248	0.257	0.238 ± 0.015	0.189	0.129	0.253	0.190 ± 0.036
	48	0.666	0.542	0.573	0.594 ± 0.037	0.486	0.523	0.672	0.560 ± 0.057
	72	1.114	0.829	0.820	0.921 ± 0.097	0.635	0.903	1.006	0.848 ± 0.111
	96	1.564	1.364	1.077	1.335 ± 0.141	0.710	1.139	1.336	1.062 ± 0.185
Total	6	0.148	0.130	0.187	0.155 ± 0.017	0.071	0.084	0.084	0.080 ± 0.004
	12	0.473	0.637	0.639	0.583 ± 0.055	0.321	0.237	0.593	0.384 ± 0.107
	24	1.319	1.395	1.565	1.426 ± 0.073	1.173	0.851	1.372	1.132 ± 0.152
	48	3.310	3.155	3.050	3.172 ± 0.076	2.554	3.135	3.166	2.952 ± 0.199
	72	5.300	4.639	4.129	4.689 ± 0.339	3.005	4.760	4.978	4.248 ± 0.625
	96	6.979	6.387	5.085	6.150 ± 0.559	3.268	5.735	6.190	5.064 ± 0.908

Table III-13. Radioactivity in Blood and Tissue of Male Fischer 344 Rats at 96 h Following Dermal Treatment with ^{14}C-Labeled 4,4'-MDA (4.0 mg/Rat): Continuous Application versus Washing at 24 h

	µg equivalents/g or mL							
	Continuous				24-h Wash			
Tissue	No. 46	No. 47	No. 48	Mean ± S.E.	No. 43	No. 44	No. 45	Mean ± S.E.
Blood	0.079	0.112	0.055	0.082 ± 0.017	0.016	0.047	0.056	0.040 ± 0.012
Plasma	0.088	0.134	0.066	0.096 ± 0.020	0.016	0.050	0.067	0.044 ± 0.015
RBCs	0.051	0.070	0.037	0.053 ± 0.010	0.011	0.028	0.033	0.024 ± 0.007
Liver	0.494	0.686	0.399	0.526 ± 0.084	0.167	0.427	0.446	0.347 ± 0.090
Kidneys	0.159	0.316	0.104	0.193 ± 0.064	0.030	0.102	0.121	0.084 ± 0.028
Lungs	0.042	0.069	0.051	0.054 ± 0.008	0.002	0.031	0.034	0.022 ± 0.010
Brain	0.006	0.022	0.006	0.011 ± 0.005	0.001	0.002	0.006	0.003 ± 0.002
Spleen	0.034	0.042	0.016	0.031 ± 0.008	0.002	0.019	0.018	0.013 ± 0.006
Testes	0.032	0.061	0.022	0.038 ± 0.012	0.001	0.014	0.030	0.015 ± 0.008
Adrenals	0.197	0.173	0.083	0.151 ± 0.035	0.037	0.046	0.057	0.047 ± 0.006
Bladder	0.001	0.000	0.000	0.000 ± 0.000	0.000	0.000	0.000	0.000 ± 0.000
Muscle	0.019	0.037	0.011	0.022 ± 0.008	0.002	0.011	0.014	0.009 ± 0.004
Fat	0.007	0.011	0.002	0.007 ± 0.003	0.002	0.002	0.002	0.002 ± 0.000
GI tract	0.256	0.572	0.222	0.350 ± 0.111	0.042	0.205	0.148	0.132 ± 0.048
Nontreated skin	0.061	0.090	0.068	0.073 ± 0.009	0.054	0.036	0.043	0.044 ± 0.005

Table III-13 (continued)

	Percent of administered dose							
	Continuous				24-h Wash			
Tissue/excretum	No. 46	No. 47	No. 48	Mean ± S.E.	No. 43	No. 44	No. 45	Mean ± S.E.
Blood[a]	0.027	0.039	0.020	0.029 ± 0.006	0.006	0.017	0.020	0.014 ± 0.004
Plasma[a,b]	0.018	0.028	0.014	0.020 ± 0.004	0.004	0.011	0.014	0.010 ± 0.003
RBCs[a,b]	0.007	0.010	0.005	0.007 ± 0.001	0.002	0.004	0.005	0.004 ± 0.001
Liver	0.101	0.137	0.086	0.108 ± 0.015	0.035	0.075	0.094	0.068 ± 0.017
Kidneys	0.008	0.014	0.005	0.009 ± 0.003	0.001	0.004	0.005	0.003 ± 0.001
Lungs	0.001	0.001	0.001	0.001 ± 0.000	0.000	0.001	0.001	0.001 ± 0.000
Brain	0.000	0.001	0.000	0.000 ± 0.000	0.000	0.000	0.000	0.000 ± 0.000
Spleen	0.000	0.000	0.000	0.000 ± 0.000	0.000	0.000	0.000	0.000 ± 0.000
Testes	0.002	0.004	0.002	0.003 ± 0.001	0.000	0.001	0.002	0.001 ± 0.001
Adrenals	0.000	0.000	0.000	0.000 ± 0.000	0.000	0.000	0.000	0.000 ± 0.000
Bladder	0.000	0.000	0.000	0.000 ± 0.000	0.000	0.000	0.000	0.000 ± 0.000
Muscle[a]	0.037	0.071	0.022	0.043 ± 0.014	0.004	0.022	0.027	0.018 ± 0.007
GI tract	0.144	0.266	0.134	0.181 ± 0.042	0.023	0.089	0.082	0.065 ± 0.021
Nontreated skin[a]	0.048	0.071	0.055	0.058 ± 0.007	0.042	0.028	0.035	0.035 ± 0.004
Urine	5.415	5.023	4.008	4.815 ± 0.419	2.558	4.596	4.854	4.003 ± 0.726
Feces	1.564	1.364	1.077	1.335 ± 0.141	0.710	1.139	1.336	1.062 ± 0.185
Dose wash	65.513	64.590	57.233	62.445 ± 2.620	80.807	81.869	71.073	77.916 ± 3.435
Application area	22.702	17.146	32.136	23.995 ± 4.375	3.808	6.886	16.084	8.926 ± 3.688
Recovery[b]	95.562	88.727	94.779	93.023 ± 2.160	87.994	94.727	93.613	92.111 ± 2.084

[a] Calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[b] Individual blood components and fat are not included in recovery estimates.

Table III-14. Recovery of Radioactivity in Blood, Tissue, and Excreta at 96 h Following Dermal Treatment of Male Fischer 344 Rats with ^{14}C-Labeled 4,4'-MDA (4.0 mg/Rat): Continuous Application versus Washing at 24 h

Tissue/ excretum	Percent of dose							
	Continuous				24-h Wash			
	No. 46	No. 47	No. 48	Mean ± S.E.	No. 43	No. 44	No. 45	Mean ± S.E.
Blood	0.027	0.039	0.020	0.029 ± 0.006	0.006	0.017	0.020	0.014 ± 0.004
Tissue	0.197	0.299	0.171	0.222 ± 0.039	0.082	0.131	0.164	0.126 ± 0.024
GI tract	0.144	0.266	0.134	0.181 ± 0.042	0.023	0.089	0.082	0.065 ± 0.021
Urine	5.415	5.023	4.008	4.815 ± 0.419	2.558	4.596	4.854	4.003 ± 0.726
Feces	1.564	1.364	1.077	1.335 ± 0.141	0.710	1.139	1.336	1.062 ± 0.185
Total absorbed	7.347	6.991	5.410	6.583 ± 0.595	3.379	5.972	6.456	5.269 ± 0.955
Dose wash	65.513	64.590	57.233	62.445 ± 2.620	80.807	81.869	71.073	77.916 ± 3.435
Application area	22.702	17.146	32.136	23.995 ± 4.375	3.808	6.886	16.084	8.926 ± 3.688
Recovery	95.562	88.727	94.779	93.023 ± 2.160	87.994	94.727	93.613	92.111 ± 2.084

APPENDIX IV

GUINEA PIG STUDIES
INDIVIDUAL ANIMAL DATA

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Table IV-1. Urinary and Fecal Excretion of Radioactivity in Male Hartley Guinea Pigs Treated Intravenously with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)

Excretum	Time after dosing (h)	Percent of dose No. 67	 No. 68	 No. 69	 Mean ± S.E.	Cumulative percent of dose No. 67	 No. 68	 No. 69	 Mean ± S.E.
Urine	6	7.890	4.909	6.489	6.429 ± 0.861	7.890	4.909	6.489	6.429 ± 0.861
	12	12.521	13.974	19.493	15.329 ± 2.124	20.411	18.883	25.982	21.759 ± 2.157
	24	12.912	6.258	7.496	8.889 ± 2.043	33.323	25.141	33.478	30.647 ± 2.754
	48	4.176	2.925	2.831	3.311 ± 0.434	37.499	28.066	36.309	33.958 ± 2.966
	72	0.604	0.675	0.763	0.681 ± 0.046	38.103	28.741	37.072	34.639 ± 2.964
	96	0.302	0.213	0.522	0.346 ± 0.092	38.405	28.954	37.594	34.984 ± 3.024
Feces	6	2.122	1.589	1.626	1.779 ± 0.172	2.122	1.589	1.626	1.779 ± 0.172
	12	0.079	7.006	5.054	4.046 ± 2.062	2.201	8.595	6.680	5.825 ± 1.895
	24	26.809	30.216	24.841	27.289 ± 1.570	29.010	38.811	31.521	33.114 ± 2.939
	48	22.212	18.073	13.711	17.999 ± 2.454	51.222	56.884	45.232	51.113 ± 3.364
	72	3.804	4.349	4.063	4.072 ± 0.157	55.026	61.233	49.295	55.185 ± 3.447
	96	1.453	1.635	0.708	1.265 ± 0.284	56.479	62.868	50.003	56.450 ± 3.714
Total	6	10.012	6.498	8.115	8.208 ± 1.015	10.012	6.498	8.115	8.208 ± 1.015
	12	12.600	20.980	24.547	19.376 ± 3.541	22.612	27.478	32.662	27.584 ± 2.902
	24	39.721	36.474	32.337	36.177 ± 2.137	62.333	63.952	64.999	63.761 ± 0.775
	48	26.388	20.998	16.542	21.309 ± 2.847	88.721	84.950	81.541	85.071 ± 2.074
	72	4.408	5.024	4.826	4.753 ± 0.182	93.129	89.974	86.367	89.823 ± 1.953
	96	1.755	1.848	1.230	1.611 ± 0.192	94.884	91.822	87.597	91.434 ± 2.112

Table IV-2. Radioactivity in Blood, Tissue, and Excreta at 96 h Following Intravenous Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)

Tissue/	µg equivalents/g or mL				Percent of dose			
excretum	No. 67	No. 68	No. 69	Mean ± S.E.	No. 67	No. 68	No. 69	Mean ± S.E.
Blood[a]	0.274	0.196	0.168	0.213 ± 0.032	0.676	0.525	0.449	0.550 ± 0.067
Plasma[a,b]	0.266	0.169	0.169	0.201 ± 0.032	0.394	0.271	0.271	0.312 ± 0.041
RBCs[a,b]	0.216	0.199	0.196	0.204 ± 0.006	0.213	0.213	0.210	0.212 ± 0.001
Liver	0.931	0.684	0.687	0.767 ± 0.082	1.603	1.813	1.622	1.679 ± 0.067
Kidneys	0.320	0.232	0.180	0.244 ± 0.041	0.116	0.102	0.108	0.109 ± 0.004
Lungs	0.203	0.314	0.640	0.386 ± 0.131	0.045	0.073	0.176	0.098 ± 0.040
Brain	0.005	0.003	0.003	0.004 ± 0.001	0.003	0.002	0.002	0.002 ± 0.000
Spleen	1.071	1.376	1.111	1.186 ± 0.096	0.050	0.060	0.080	0.063 ± 0.009
Testes	0.030	0.023	0.012	0.022 ± 0.005	0.003	0.002	0.001	0.002 ± 0.001
Adrenals	0.178	0.168	0.228	0.191 ± 0.019	0.003	0.002	0.004	0.003 ± 0.001
Bladder	0.018	0.007	0.012	0.012 ± 0.003	0.001	0.000	0.000	0.000 ± 0.000
Muscle[a]	0.015	0.010	0.006	0.010 ± 0.003	0.212	0.153	0.092	0.152 ± 0.035
Fat[b]	0.030	0.029	0.021	0.027 ± 0.003	-	-	-	-
GI tract	0.111	0.062	0.067	0.080 ± 0.016	0.699	0.482	0.641	0.607 ± 0.065
Skin[a]	0.056	0.043	0.041	0.047 ± 0.005	0.314	0.263	0.249	0.275 ± 0.020
Urine	-	-	-	-	38.405	28.954	37.594	34.984 ± 3.024
Feces	-	-	-	-	56.479	62.868	50.003	56.450 ± 3.714
Recovery[b]	-	-	-	-	98.609	95.299	91.021	94.976 ± 2.196

[a] Percent of dose calculations are based on 7%, 16%, and 40% of body weight for blood, skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[b] Individual blood components and fat are not included in recovery estimates.

Table IV-3. Recovery of Radioactivity in Blood, Tissue, and Excreta at 96 h Following Intravenous Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)

Tissue/	Percent of dose			
excretum	No. 67	No. 68	No. 69	Mean ± S.E.
Blood	0.676	0.525	0.449	0.550 ± 0.067
Tissue	2.350	2.470	2.334	2.385 ± 0.043
GI tract	0.699	0.482	0.641	0.607 ± 0.065
Urine	38.405	28.954	37.594	34.984 ± 3.024
Feces	56.479	62.868	50.003	56.450 ± 3.714
Recovery	98.609	95.299	91.021	94.976 ± 2.196

Table IV-4. Urinary and Fecal Excretion of Radioactivity in Male Hartley Guinea Pigs Treated Dermally with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)

Excretum	Time after dosing (h)	24-h sacrifice				96-h sacrifice			
		No. 76	No. 77	No. 78	Mean ± S.E.	No. 73	No. 74	No. 75	Mean ± S.E.
		Percent of dose							
Urine	6	0.090	0.701	5.286	2.026 ± 1.640	0.311	0.215	0.124	0.217 ± 0.054
	12	1.194	6.381	2.701	3.425 ± 1.541	1.761	0.820	1.319	1.300 ± 0.272
	24	1.492	3.352	2.218	2.354 ± 0.541	2.417	1.719	4.259	2.798 ± 0.758
	48					4.364	1.875	3.730	3.323 ± 0.747
	72					1.503	0.799	2.185	1.496 ± 0.400
	96					2.151	0.628	1.214	1.331 ± 0.444
Feces	6	0.014	0.044	0.093	0.050 ± 0.023	0.031	0.048	0.012	0.030 ± 0.010
	12	0.657	1.704	1.097	1.153 ± 0.304	0.127	1.257	0.924	0.769 ± 0.335
	24	2.968	7.721	2.808	4.499 ± 1.612	3.700	4.958	2.963	3.874 ± 0.582
	48					7.490	5.027	9.296	7.271 ± 1.237
	72					3.601	2.558	4.563	3.574 ± 0.579
	96					2.326	1.548	2.441	2.105 ± 0.280
Total	6	0.104	0.745	5.379	2.076 ± 1.662	0.342	0.263	0.136	0.247 ± 0.060
	12	1.851	8.085	3.798	4.578 ± 1.841	1.888	2.077	2.243	2.069 ± 0.103
	24	4.460	11.073	5.026	6.853 ± 2.116	6.117	6.677	7.222	6.672 ± 0.319
	48					11.854	6.902	13.026	10.594 ± 1.877
	72					5.104	3.357	6.748	5.070 ± 0.979
	96					4.477	2.176	3.655	3.436 ± 0.673
		Cumulative percent of dose							
Urine	6	0.090	0.701	5.286	2.026 ± 1.640	0.311	0.215	0.124	0.217 ± 0.054
	12	1.284	7.082	7.987	5.451 ± 2.100	2.072	1.035	1.443	1.517 ± 0.302
	24	2.776	10.434	10.205	7.805 ± 2.515	4.489	2.754	5.702	4.315 ± 0.855
	48					8.853	4.629	9.432	7.638 ± 1.514
	72					10.356	5.428	11.617	9.134 ± 1.888
	96					12.507	6.056	12.831	10.465 ± 2.206
Feces	6	0.014	0.044	0.093	0.050 ± 0.023	0.031	0.048	0.012	0.030 ± 0.010
	12	0.671	1.748	1.190	1.203 ± 0.311	0.158	1.305	0.936	0.800 ± 0.338
	24	3.639	9.469	3.998	5.702 ± 1.886	3.858	6.263	3.899	4.673 ± 0.795
	48					11.348	11.290	13.195	11.944 ± 0.626
	72					14.949	13.848	17.758	15.518 ± 1.164
	96					17.275	15.396	20.199	17.623 ± 1.397
Total	6	0.104	0.745	5.379	2.076 ± 1.662	0.342	0.263	0.136	0.247 ± 0.060
	12	1.955	8.830	9.177	6.654 ± 2.352	2.230	2.340	2.379	2.316 ± 0.045
	24	6.415	19.903	14.203	13.507 ± 3.909	8.347	9.017	9.601	8.988 ± 0.362
	48					20.201	15.919	22.627	19.582 ± 1.961
	72					25.305	19.276	29.375	24.652 ± 2.934
	96					29.782	21.452	33.030	28.088 ± 3.448

Table IV-5. Radioactivity in Blood and Tissue at 6, 24, and 96 h Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)

	µg equivalents/g or mL											
	6 h				24 h				96 h			
Tissue	No. 79	No. 80	No. 81	Mean ± S.E.	No. 76	No. 77	No. 78	Mean ± S.E.	No. 73	No. 74	No. 75	Mean ± S.E.
Blood	0.053	0.039	0.053	0.048 ± 0.005	0.049	0.083	0.044	0.059 ± 0.012	0.061	0.041	0.073	0.058 ± 0.009
Plasma	0.056	0.044	0.057	0.052 ± 0.004	0.053	0.084	0.042	0.060 ± 0.013	0.061	0.037	0.067	0.055 ± 0.009
RBCs	0.033	0.024	0.019	0.025 ± 0.004	0.033	0.058	0.035	0.042 ± 0.008	0.057	0.044	0.067	0.056 ± 0.007
Liver	0.295	0.233	0.227	0.252 ± 0.022	0.231	0.356	0.248	0.278 ± 0.039	0.266	0.089	0.217	0.191 ± 0.053
Kidneys	0.090	0.090	0.096	0.092 ± 0.002	0.100	0.171	0.101	0.124 ± 0.024	0.152	0.061	0.105	0.106 ± 0.026
Lungs	0.061	0.042	0.057	0.053 ± 0.006	0.053	0.078	0.038	0.056 ± 0.012	0.045	0.016	0.044	0.035 ± 0.010
Brain	0.043	0.020	0.028	0.030 ± 0.007	0.017	0.022	0.014	0.018 ± 0.002	0.005	0.002	0.005	0.004 ± 0.001
Spleen	0.041	0.030	0.033	0.035 ± 0.003	0.023	0.037	0.019	0.026 ± 0.005	0.027	0.010	0.015	0.017 ± 0.005
Testes	0.107	0.095	0.148	0.117 ± 0.016	0.048	0.097	0.049	0.065 ± 0.016	0.031	0.017	0.024	0.024 ± 0.004
Adrenals	0.853	0.553	1.887	1.098 ± 0.404	0.944	0.954	1.031	0.976 ± 0.027	0.668	0.472	0.403	0.514 ± 0.079
Bladder	0.011	0.004	0.005	0.006 ± 0.002	0.002	0.003	0.002	0.002 ± 0.000	0.009	0.003	0.004	0.005 ± 0.002
Muscle	0.016	0.018	0.031	0.022 ± 0.005	0.012	0.020	0.010	0.014 ± 0.003	0.060	0.015	0.004	0.026 ± 0.017
Fat	0.031	0.031	0.026	0.029 ± 0.002	0.013	0.016	0.014	0.014 ± 0.001	0.013	0.009	0.012	0.011 ± 0.001
GI tract	0.288	0.257	0.281	0.275 ± 0.009	0.509	0.999	0.509	0.672 ± 0.163	0.154	0.070	0.089	0.104 ± 0.025
Nontreated skin	0.055	0.039	0.067	0.054 ± 0.008	0.029	0.092	0.026	0.049 ± 0.022	0.025	0.026	0.075	0.042 ± 0.017

Table IV-5 (continued)

Tissue/ excretum	Percent of administered dose											
	6 h				24 h				96 h			
	No. 79	No. 80	No. 81	Mean ± S.E.	No. 76	No. 77	No. 78	Mean ± S.E.	No. 73	No. 74	No. 75	Mean ± S.E.
Blood[a]	0.119	0.085	0.148	0.117 ± 0.018	0.121	0.199	0.104	0.141 ± 0.029	0.154	0.095	0.177	0.142 ± 0.024
Plasma[a,b]	0.076	0.057	0.097	0.077 ± 0.012	0.079	0.120	0.060	0.086 ± 0.018	0.093	0.052	0.098	0.081 ± 0.015
RBCs[a,b]	0.030	0.021	0.021	0.024 ± 0.003	0.033	0.055	0.033	0.040 ± 0.007	0.058	0.041	0.065	0.055 ± 0.007
Liver	0.418	0.387	0.380	0.395 ± 0.012	0.401	0.622	0.409	0.477 ± 0.072	0.528	0.243	0.436	0.402 ± 0.084
Kidneys	0.044	0.041	0.046	0.044 ± 0.001	0.038	0.064	0.031	0.044 ± 0.010	0.060	0.032	0.053	0.048 ± 0.008
Lungs	0.016	0.011	0.013	0.013 ± 0.001	0.013	0.017	0.008	0.013 ± 0.003	0.010	0.005	0.012	0.009 ± 0.002
Brain	0.020	0.009	0.013	0.014 ± 0.003	0.007	0.009	0.006	0.007 ± 0.001	0.002	0.001	0.003	0.002 ± 0.001
Spleen	0.002	0.001	0.002	0.002 ± 0.000	0.001	0.002	0.001	0.001 ± 0.000	0.001	0.001	0.001	0.001 ± 0.000
Testes	0.011	0.013	0.025	0.016 ± 0.004	0.005	0.009	0.005	0.006 ± 0.001	0.003	0.001	0.003	0.002 ± 0.001
Adrenals	0.014	0.009	0.040	0.021 ± 0.010	0.013	0.016	0.016	0.015 ± 0.001	0.012	0.008	0.007	0.009 ± 0.002
Bladder	0.000	0.000	0.000	0.000 ± 0.000	0.000	0.000	0.000	0.000 ± 0.000	0.000	0.000	0.000	0.000 ± 0.000
Muscle[a]	0.201	0.218	0.499	0.306 ± 0.097	0.163	0.266	0.129	0.186 ± 0.041	0.864	0.195	0.049	0.369 ± 0.251
GI tract	1.679	1.294	1.549	1.507 ± 0.113	2.301	4.624	1.461	2.795 ± 0.946	0.870	0.402	0.493	0.588 ± 0.143
Nontreated skin[a]	0.282	0.194	0.428	0.301 ± 0.068	0.164	0.502	0.141	0.269 ± 0.117	0.142	0.140	0.415	0.232 ± 0.091
Urine	0.292	0.548	0.216	0.352 ± 0.100	2.776	10.434	10.205	7.805 ± 2.515	12.507	6.056	12.831	10.465 ± 2.206
Feces	0.116	0.046	0.133	0.098 ± 0.027	3.639	9.469	3.998	5.702 ± 1.886	17.275	15.396	20.199	17.623 ± 1.397
Dose wash	83.530	78.900	79.215	80.548 ± 1.494	68.980	44.605	62.740	58.775 ± 7.310	38.805	49.095	34.635	40.845 ± 4.297
Application area	13.225	9.481	11.572	11.426 ± 1.083	14.891	17.700	11.721	14.771 ± 1.727	26.077	33.843	28.294	29.405 ± 2.310
Recovery[b]	99.969	91.237	94.279	95.162 ± 2.559	93.513	88.538	90.975	91.009 ± 1.436	97.310	105.513	97.608	100.144 ± 2.686

[a] Calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[b] Individual blood components and fat are not included in recovery estimates.

Table IV-6. Recovery of Radioactivity in Blood, Tissue, and Excreta at 6, 24, or 96 h Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig)

Tissue/ excretum	Percent of dose											
	6 h				24 h				96 h			
	No. 79	No. 80	No. 81	Mean ± S.E.	No. 76	No. 77	No. 78	Mean ± S.E.	No. 73	No. 74	No. 75	Mean ± S.E.
Blood	0.119	0.085	0.148	0.117 ± 0.018	0.121	0.199	0.104	0.141 ± 0.029	0.154	0.095	0.177	0.142 ± 0.024
Tissue	1.008	0.883	1.446	1.112 ± 0.171	0.805	1.507	0.746	1.019 ± 0.244	1.622	0.626	0.979	1.076 ± 0.292
GI tract	1.679	1.294	1.549	1.507 ± 0.113	2.301	4.624	1.461	2.795 ± 0.946	0.870	0.402	0.493	0.588 ± 0.143
Urine	0.292	0.548	0.216	0.352 ± 0.100	2.776	10.434	10.205	7.805 ± 2.515	12.507	6.056	12.831	10.465 ± 2.206
Feces	0.116	0.046	0.133	0.098 ± 0.027	3.639	9.469	3.998	5.702 ± 1.886	17.275	15.396	20.199	17.623 ± 1.397
Total absorbed	3.214	2.855	3.492	3.187 ± 0.184	9.516	26.233	16.514	17.421 ± 4.847	32.428	22.575	34.679	29.894 ± 3.717
Dose wash	83.530	78.900	79.215	80.548 ± 1.494	68.980	44.605	62.740	58.775 ± 7.310	38.805	49.095	34.635	40.845 ± 4.297
Application area	13.225	9.481	11.572	11.426 ± 1.083	14.891	17.700	11.721	14.771 ± 1.727	26.077	33.843	28.294	29.405 ± 2.310
Recovery	99.969	91.237	94.279	95.162 ± 2.559	93.513	88.538	90.975	91.009 ± 1.436	97.310	105.513	97.608	100.144 ± 2.686

Table IV-7. Radioactivity in Blood, Tissue, and Excreta 6 h Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig): Nonoccluded Application Area

Tissue/ excretum	μg equivalents/g or mL				Percent of dose			
	No. 82	No. 83	No. 84	Mean ± S.E.	No. 82	No. 83	No. 84	Mean ± S.E.
Blood[a]	0.052	0.040	0.044	0.045 ± 0.004	0.146	0.086	0.120	0.117 ± 0.017
Plasma[a,b]	0.049	0.047	0.043	0.046 ± 0.002	0.083	0.061	0.070	0.071 ± 0.006
RBCs[a,b]	0.043	0.021	0.031	0.032 ± 0.006	0.048	0.018	0.034	0.033 ± 0.009
Liver	0.185	0.259	0.226	0.223 ± 0.021	0.304	0.497	0.299	0.367 ± 0.065
Kidneys	0.198	0.087	0.284	0.190 ± 0.057	0.100	0.049	0.107	0.085 ± 0.018
Lungs	0.049	0.059	0.047	0.052 ± 0.004	0.012	0.015	0.010	0.012 ± 0.001
Brain	0.038	0.027	0.025	0.030 ± 0.004	0.018	0.013	0.012	0.014 ± 0.002
Spleen	0.040	0.031	0.046	0.039 ± 0.004	0.002	0.002	0.002	0.002 ± 0.000
Testes	0.112	0.100	0.085	0.099 ± 0.008	0.015	0.013	0.013	0.013 ± 0.001
Adrenals	1.079	0.650	0.760	0.830 ± 0.129	0.020	0.011	0.011	0.014 ± 0.003
Bladder	0.011	0.006	0.004	0.007 ± 0.002	0.000	0.000	0.000	0.000 ± 0.000
Muscle[a]	0.018	0.016	0.021	0.018 ± 0.001	0.282	0.190	0.319	0.264 ± 0.038
Fat[b]	0.014	0.019	0.013	0.015 ± 0.002	-	-	-	-
GI tract	0.110	0.286	0.192	0.196 ± 0.051	0.859	1.846	1.117	1.274 ± 0.300
Nontreated skin[a]	0.037	0.034	0.056	0.042 ± 0.007	0.239	0.167	0.349	0.252 ± 0.053
Urine	-	-	-	-	0.116	0.147	1.549	0.604 ± 0.473
Feces	-	-	-	-	0.088	0.039	0.235	0.121 ± 0.059
Dose wash	-	-	-	-	88.680	84.975	88.718	87.458 ± 1.241
Application area	-	-	-	-	8.448	10.525	9.147	9.373 ± 0.610
Recovery[b]	-	-	-	-	99.329	98.575	102.005	99.970 ± 1.041

[a] Percent of dose calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively.
Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.
[b] Individual blood components and fat are not included in recovery estimates.

Table IV-8. Recovery of Radioactivity in Blood, Tissue, and Excreta at 6 h Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig): Nonoccluded Application Area

Tissue/ excretum	Percent of dose			
	No. 82	No. 83	No. 84	Mean ± S.E.
Blood	0.146	0.086	0.120	0.117 ± 0.017
Tissue	0.992	0.957	1.119	1.023 ± 0.049
GI tract	0.859	1.846	1.117	1.274 ± 0.300
Urine	0.116	0.147	1.549	0.604 ± 0.473
Feces	0.088	0.039	0.235	0.121 ± 0.059
Total absorbed	2.201	3.075	4.140	3.139 ± 0.561
Dose wash	88.680	84.975	88.718	87.458 ± 1.241
Application area	8.448	10.525	9.147	9.373 ± 0.610
Recovery	99.329	98.575	102.005	99.970 ± 1.041

Table IV-9. Urinary and Fecal Excretion of Radioactivity in Male Hartley Guinea Pigs Treated Dermally with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig): Application Area Washed at 24 h

Excretum	Time after dosing (h)	Percent of dose				Cumulative percent of dose			
		No. 70	No. 71	No. 72	Mean ± S.E.	No. 70	No. 71	No. 72	Mean ± S.E.
Urine	6	0.168	0.129	0.225	0.174 ± 0.028	0.168	0.129	0.225	0.174 ± 0.028
	12	1.191	1.943	1.456	1.530 ± 0.220	1.359	2.072	1.681	1.704 ± 0.206
	24	2.012	2.527	2.312	2.284 ± 0.149	3.371	4.599	3.993	3.988 ± 0.355
	48	1.788	1.620	2.961	2.123 ± 0.422	5.159	6.219	6.954	6.111 ± 0.521
	72	0.325	0.205	0.256	0.262 ± 0.035	5.484	6.424	7.210	6.373 ± 0.499
	96	0.253	0.143	0.179	0.192 ± 0.032	5.737	6.567	7.389	6.564 ± 0.477
Feces	6	0.045	0.018	0.010	0.024 ± 0.011	0.045	0.018	0.010	0.024 ± 0.011
	12	1.308	0.971	1.139	1.139 ± 0.097	1.353	0.989	1.149	1.164 ± 0.105
	24	3.988	5.707	2.276	3.990 ± 0.990	5.341	6.696	3.425	5.154 ± 0.949
	48	4.771	4.439	5.597	4.936 ± 0.344	10.112	11.135	9.022	10.090 ± 0.610
	72	0.494	0.478	0.518	0.497 ± 0.012	10.606	11.613	9.540	10.586 ± 0.599
	96	0.442	0.200	0.258	0.300 ± 0.073	11.048	11.813	9.798	10.886 ± 0.587
Total	6	0.213	0.147	0.235	0.198 ± 0.026	0.213	0.147	0.235	0.198 ± 0.026
	12	2.499	2.914	2.595	2.669 ± 0.125	2.712	3.061	2.830	2.868 ± 0.102
	24	6.000	8.234	4.588	6.274 ± 1.061	8.712	11.295	7.418	9.142 ± 1.140
	48	6.559	6.059	8.558	7.059 ± 0.763	15.271	17.354	15.976	16.200 ± 0.612
	72	0.819	0.683	0.774	0.759 ± 0.040	16.090	18.037	16.750	16.959 ± 0.572
	96	0.695	0.343	0.437	0.492 ± 0.105	16.785	18.380	17.187	17.451 ± 0.479

Table IV-10. Radioactivity in Blood, Tissue, and Excreta at 96 h Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig): Application Area Washed at 24 h

Tissue/ excretum	μg equivalents/g or mL				Percent of dose			
	No. 70	No. 71	No. 72	Mean ± S.E.	No. 70	No. 71	No. 72	Mean ± S.E.
Blood[a]	0.032	0.031	0.035	0.033 ± 0.001	0.078	0.078	0.082	0.079 ± 0.001
Plasma[a,b]	0.026	0.020	0.029	0.025 ± 0.003	0.039	0.030	0.041	0.037 ± 0.003
RBCs[a,b]	0.034	0.038	0.042	0.038 ± 0.002	0.034	0.038	0.039	0.037 ± 0.002
Liver	0.098	0.129	0.096	0.108 ± 0.011	0.194	0.272	0.190	0.219 ± 0.027
Kidneys	0.057	0.057	0.060	0.058 ± 0.001	0.023	0.025	0.023	0.024 ± 0.001
Lungs	0.016	0.015	0.017	0.016 ± 0.001	0.004	0.003	0.004	0.004 ± 0.000
Brain	0.002	0.002	0.001	0.002 ± 0.000	0.001	0.001	0.000	0.001 ± 0.000
Spleen	0.007	0.008	0.011	0.009 ± 0.001	0.000	0.001	0.000	0.000 ± 0.000
Testes	0.006	0.005	0.003	0.005 ± 0.001	0.000	0.001	0.000	0.000 ± 0.000
Adrenals	0.072	0.065	0.061	0.066 ± 0.003	0.001	0.001	0.001	0.001 ± 0.000
Bladder	0.004	0.003	0.003	0.003 ± 0.000	0.000	0.000	0.000	0.000 ± 0.000
Muscle[a]	0.002	0.004	0.003	0.003 ± 0.001	0.021	0.051	0.034	0.035 ± 0.009
Fat[b]	0.012	0.005	0.008	0.008 ± 0.002	-	-	-	-
GI tract	0.026	0.014	0.015	0.018 ± 0.004	0.191	0.085	0.083	0.120 ± 0.036
Nontreated skin[a]	0.010	0.006	0.010	0.009 ± 0.001	0.054	0.032	0.051	0.046 ± 0.007
Urine	-	-	-	-	5.737	6.567	7.389	6.564 ± 0.477
Feces	-	-	-	-	11.048	11.813	9.798	10.886 ± 0.587
Dose wash	-	-	-	-	60.535	60.925	63.880	61.780 ± 1.056
Application area	-	-	-	-	19.255	15.285	15.201	16.580 ± 1.338
Recovery[b]	-	-	-	-	97.142	95.140	96.736	96.339 ± 0.611

[a] Percent of dose calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively.
Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[b] Individual blood components and fat are not included in recovery estimates.

Table IV-11. Recovery of Radioactivity in Blood, Tissue, and Excreta at 96 h Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (1.0 mg/Guinea Pig): Application Area Washed at 24 h

Tissue/ excretum	Percent of dose			
	No. 70	No. 71	No. 72	Mean ± S.E.
Blood	0.078	0.078	0.082	0.079 ± 0.001
Tissue	0.298	0.387	0.303	0.329 ± 0.029
GI tract	0.191	0.085	0.083	0.120 ± 0.036
Urine	5.737	6.567	7.389	6.564 ± 0.477
Feces	11.048	11.813	9.798	10.886 ± 0.587
Total absorbed	17.352	18.930	17.655	17.979 ± 0.483
Dose wash	60.535	60.925	63.880	61.780 ± 1.056
Application area	19.255	15.285	15.201	16.580 ± 1.338
Recovery	97.142	95.140	96.736	96.339 ± 0.611

Table IV-12. Urinary and Fecal Excretion of Radioactivity in Male Hartley Guinea Pigs Treated Dermally with ^{14}C-Labeled 4,4'-MDA (10.0 mg/Guinea Pig): Continuous Application versus Washing at 24 h

		Percent of dose						
		Continuous			24-h Wash			
Excretum	Time after dosing (h)	No. 64	No. 65	Average	No. 61	No. 62	No. 63	Mean ± S.E.
Urine	6	0.058	0.021	0.040	0.023	0.021	0.099	0.048 ± 0.026
	12	0.183	0.339	0.261	0.178	0.156	0.132	0.155 ± 0.013
	24	0.326	0.159	0.243	0.354	0.265	0.245	0.288 ± 0.034
	48	0.525	0.277	0.401	0.318	1.011	0.481	0.603 ± 0.209
	72	0.823	0.541	0.682	0.087	0.352	0.337	0.259 ± 0.086
	96	0.511	1.741	1.126	0.066	0.266	0.228	0.187 ± 0.061
Feces	6	0.005	0.012	0.009	0.003	0.004	0.002	0.003 ± 0.001
	12	0.018	0.046	0.032	0.064	0.056	0.051	0.057 ± 0.004
	24	0.673	0.724	0.699	0.601	0.435	0.511	0.516 ± 0.048
	48	1.255	0.675	0.965	0.470	1.081	0.778	0.776 ± 0.176
	72	1.146	0.562	0.854	0.269	0.720	0.306	0.432 ± 0.145
	96	1.072	1.048	1.060	0.162	0.612	0.406	0.393 ± 0.130
Total	6	0.063	0.033	0.048	0.026	0.025	0.101	0.051 ± 0.025
	12	0.201	0.385	0.293	0.242	0.212	0.183	0.212 ± 0.017
	24	0.999	0.883	0.941	0.955	0.700	0.756	0.804 ± 0.077
	48	1.780	0.952	1.366	0.788	2.092	1.259	1.380 ± 0.381
	72	1.969	1.103	1.536	0.356	1.072	0.643	0.690 ± 0.208
	96	1.583	2.789	2.186	0.228	0.878	0.634	0.580 ± 0.190
		Cumulative percent of dose						
Urine	6	0.058	0.021	0.040	0.023	0.021	0.099	0.048 ± 0.026
	12	0.241	0.360	0.301	0.201	0.177	0.231	0.203 ± 0.016
	24	0.567	0.519	0.543	0.555	0.442	0.476	0.491 ± 0.033
	48	1.092	0.796	0.944	0.873	1.453	0.957	1.094 ± 0.181
	72	1.915	1.337	1.626	0.960	1.805	1.294	1.353 ± 0.246
	96	2.426	3.078	2.752	1.026	2.071	1.522	1.540 ± 0.302
Feces	6	0.005	0.012	0.009	0.003	0.004	0.002	0.003 ± 0.001
	12	0.023	0.058	0.041	0.067	0.060	0.053	0.060 ± 0.004
	24	0.696	0.782	0.739	0.668	0.495	0.564	0.576 ± 0.050
	48	1.951	1.457	1.704	1.138	1.576	1.342	1.352 ± 0.127
	72	3.097	2.019	2.558	1.407	2.296	1.648	1.784 ± 0.265
	96	4.169	3.067	3.618	1.569	2.908	2.054	2.177 ± 0.391
Total	6	0.063	0.033	0.048	0.026	0.025	0.101	0.051 ± 0.025
	12	0.264	0.418	0.341	0.268	0.237	0.284	0.263 ± 0.014
	24	1.263	1.301	1.282	1.223	0.937	1.040	1.067 ± 0.084
	48	3.043	2.253	2.648	2.011	3.029	2.299	2.446 ± 0.303
	72	5.012	3.356	4.184	2.367	4.101	2.942	3.137 ± 0.510
	96	6.595	6.145	6.370	2.595	4.979	3.576	3.717 ± 0.692

Table IV-13. Radioactivity in Blood and Tissue at 96 h Following Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (10.0 mg/Guinea Pigs): Continuous Application versus Washing at 24 h

	µg equivalents/g or mL						
	Continuous			24-h Wash			
Tissue	No. 64	No. 65	Average	No. 61	No. 62	No. 63	Mean ± S.E.
Blood	0.179	0.135	0.157	0.049	0.146	0.072	0.089 ± 0.029
Plasma	0.196	0.149	0.173	0.043	0.149	0.069	0.087 ± 0.032
RBCs	0.147	0.100	0.124	0.051	0.126	0.059	0.079 ± 0.024
Liver	0.654	0.524	0.589	0.076	0.374	0.327	0.259 ± 0.093
Kidneys	0.343	0.283	0.313	0.054	0.246	0.156	0.152 ± 0.056
Lungs	0.111	0.122	0.117	0.014	0.026	0.039	0.026 ± 0.007
Brain	0.012	0.006	0.009	0.001	0.009	0.005	0.005 ± 0.002
Spleen	0.056	0.028	0.042	0.006	0.020	0.016	0.014 ± 0.004
Testes	0.115	0.749	0.432	0.019	0.042	0.042	0.034 ± 0.008
Adrenals	2.537	1.812	2.175	0.156	1.440	0.577	0.724 ± 0.378
Bladder	0.011	0.005	0.008	0.042	0.016	0.002	0.020 ± 0.012
Muscle	0.027	0.045	0.036	0.008	0.015	0.036	0.020 ± 0.008
Fat	0.076	0.035	0.056	0.010	0.007	0.015	0.011 ± 0.002
GI tract	0.692	1.077	0.885	0.072	0.549	0.603	0.408 ± 0.169
Nontreated skin	0.609	0.191	0.400	0.044	0.097	0.085	0.075 ± 0.016

Table IV-13 (continued)

Tissue/excretum	Percent of administered dose						
	Continuous			24-h Wash			
	No. 64	No. 65	Average	No. 61	No. 62	No. 63	Mean ± S.E.
Blood[a]	0.043	0.036	0.040	0.012	0.033	0.021	0.022 ± 0.006
Plasma[a,b]	0.029	0.024	0.027	0.006	0.020	0.012	0.013 ± 0.004
RBCs[a,b]	0.014	0.011	0.013	0.005	0.012	0.007	0.008 ± 0.002
Liver	0.132	0.126	0.129	0.017	0.063	0.060	0.047 ± 0.015
Kidneys	0.013	0.013	0.013	0.002	0.010	0.006	0.006 ± 0.002
Lungs	0.002	0.003	0.003	0.000	0.001	0.001	0.001 ± 0.000
Brain	0.001	0.000	0.001	0.000	0.001	0.000	0.000 ± 0.000
Spleen	0.000	0.000	0.000	0.000	0.000	0.000	0.000 ± 0.000
Testes	0.001	0.007	0.004	0.000	0.000	0.001	0.000 ± 0.000
Adrenals	0.005	0.003	0.004	0.000	0.002	0.001	0.001 ± 0.001
Bladder	0.000	0.000	0.000	0.000	0.000	0.000	0.000 ± 0.000
Muscle[a]	0.037	0.068	0.053	0.011	0.020	0.061	0.031 ± 0.015
GI tract	0.485	0.791	0.638	0.053	0.375	0.407	0.278 ± 0.113
Nontreated skin[a]	0.332	0.116	0.224	0.024	0.050	0.058	0.044 ± 0.010
Urine	2.426	3.078	2.752	1.026	2.071	1.522	1.540 ± 0.302
Feces	4.169	3.067	3.618	1.569	2.908	2.054	2.177 ± 0.391
Dose wash	66.359	73.030	69.695	81.049	75.938	84.329	80.439 ± 2.441
Application area	16.014	11.430	13.722	5.903	10.016	5.956	7.292 ± 1.362
Recovery[b]	90.019	91.768	90.894	89.666	91.488	94.477	91.877 ± 1.402

[a] Calculations are based on 7%, 16%, and 40% of body weight for blood, nontreated skin, and muscle, respectively. Plasma and red blood cell calculations are based on 60% and 40% of blood volume, respectively.

[b] Individual blood components and fat are not included in recovery estimates.

Table IV-14. Recovery of Radioactivity in Blood, Tissue, and Excreta at 96 h Following Dermal Treatment of Male Hartley Guinea Pigs with ^{14}C-Labeled 4,4'-MDA (10.0 mg/Guinea Pig): Continuous Application versus Washing at 24 h

Tissue/ excretum	Percent of dose						
	Continuous			24-h Wash			
	No. 64	No. 65	Average	No. 61	No. 62	No. 63	Mean ± S.E.
Blood	0.043	0.036	0.040	0.012	0.033	0.021	0.022 ± 0.006
Tissue	0.523	0.336	0.430	0.054	0.147	0.188	0.130 ± 0.040
GI tract	0.485	0.791	0.638	0.053	0.375	0.407	0.278 ± 0.113
Urine	2.426	3.078	2.752	1.026	2.071	1.522	1.540 ± 0.302
Feces	4.169	3.067	3.618	1.569	2.908	2.054	2.177 ± 0.391
Total absorbed	7.646	7.308	7.477	2.781	5.534	4.192	4.169 ± 0.795
Dose wash	66.359	73.030	69.695	81.049	75.938	84.329	80.439 ± 2.441
Application area	16.014	11.430	13.722	5.903	10.016	5.956	7.292 ± 1.362
Recovery	90.019	91.768	90.894	89.666	91.488	94.477	91.877 ± 1.402

APPENDIX V

MONKEY STUDIES
INDIVIDUAL ANIMAL DATA

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Table V-1. Urinary and Fecal Excretion of Radioactivity in Male Rhesus Monkeys Treated Dermally or Intravenously with ^{14}C-Labeled 4,4'-MDA (10.0 mg/Monkey)

		Percent of dose							
		Dermal				Intravenous			
Excretum	Time after dosing (h)	No. 604	No. 619	No. 604R	Mean ± S.E.	No. 529	No. 554	No. 529R	Mean ± S.E.
Urine	6	0.069	0.475	0.038	0.194 ± 0.141	37.661	21.849	15.047	24.852 ± 6.699
	12	0.939	1.087	0.415	0.814 ± 0.204	21.258	2.242	39.804	21.101 ± 10.843
	24	1.919	5.308	1.277	2.835 ± 1.250	15.641	16.699	20.868	17.736 ± 1.596
	48	9.984	5.436	6.659	7.360 ± 1.359	4.850	35.730	6.409	15.663 ± 10.044
	72	5.633	1.450	4.659	3.914 ± 1.264	0.862	6.594	1.931	3.129 ± 1.760
	96	2.370	0.687	1.853	1.637 ± 0.498	0.360	1.305	0.686	0.784 ± 0.277
	120	1.305	0.362	1.043	0.903 ± 0.281	0.257	0.418	0.433	0.369 ± 0.056
	144	1.018	0.348	0.557	0.641 ± 0.198	0.209	0.206	0.381	0.265 ± 0.058
	168	0.635	0.183	0.773	0.530 ± 0.178	0.165	0.322	0.829	0.439 ± 0.200
Feces	6	0.004	-[a]	0.003	0.004	0.034	0.174	0.003	0.070 ± 0.053
	12	0.001	-[a]	-[a]	0.001	-[a]	0.057	-[a]	0.057
	24	0.009	0.392	0.047	0.149 ± 0.122	6.887	0.093	4.820	3.933 ± 2.011
	48	0.036	0.972	0.174	0.394 ± 0.292	3.581	0.792	3.191	2.521 ± 0.872
	72	0.548	0.471	0.394	0.471 ± 0.044	0.607	5.569	0.940	2.372 ± 1.601
	96	0.589	0.089	0.436	0.371 ± 0.148	0.175	0.838	0.165	0.393 ± 0.223
	120	0.192	0.063	0.186	0.147 ± 0.042	0.134	0.149	0.252	0.178 ± 0.037
	144	0.613	0.050	0.161	0.275 ± 0.172	0.233	0.092	0.155	0.160 ± 0.041
	168	0.216	0.042	0.107	0.122 ± 0.051	0.091	0.107	0.119	0.106 ± 0.008
		Cumulative percent of dose							
Urine	6	0.069	0.475	0.038	0.194 ± 0.141	37.661	21.849	15.047	24.852 ± 6.699
	12	1.008	1.562	0.453	1.008 ± 0.320	58.919	24.091	54.851	45.954 ± 10.994
	24	2.927	6.870	1.730	3.842 ± 1.553	74.560	40.790	75.719	63.690 ± 11.455
	48	12.911	12.306	8.389	11.202 ± 1.417	79.410	76.520	82.128	79.353 ± 1.619
	72	18.544	13.756	13.048	15.116 ± 1.726	80.272	83.114	84.059	82.482 ± 1.138
	96	20.914	14.443	14.901	16.753 ± 2.085	80.632	84.419	84.745	83.265 ± 1.320
	120	22.219	14.805	15.944	17.656 ± 2.305	80.889	84.837	85.178	83.635 ± 1.376
	144	23.237	15.153	16.501	18.297 ± 2.500	81.098	85.043	85.559	83.900 ± 1.409
	168	23.872	15.336	17.274	18.827 ± 2.584	81.263	85.365	86.388	84.339 ± 1.566
Feces	6	0.004	-[a]	0.003	0.004	0.034	0.174	0.003	0.070 ± 0.053
	12	0.005	-[a]	0.003	0.004	0.034	0.231	0.003	0.089 ± 0.071
	24	0.014	0.392	0.050	0.152 ± 0.120	6.921	0.324	4.823	4.023 ± 1.946
	48	0.050	1.364	0.224	0.546 ± 0.412	10.502	1.116	8.014	6.544 ± 2.807
	72	0.598	1.835	0.618	1.017 ± 0.409	11.109	6.685	8.954	8.916 ± 1.277
	96	1.187	1.924	1.054	1.388 ± 0.271	11.284	7.523	9.119	9.309 ± 1.090
	120	1.379	1.987	1.240	1.535 ± 0.229	11.418	7.672	9.371	9.487 ± 1.083
	144	1.992	2.037	1.401	1.810 ± 0.205	11.651	7.764	9.526	9.647 ± 1.124
	168	2.208	2.079	1.508	1.932 ± 0.215	11.742	7.871	9.645	9.753 ± 1.119
Dose wash		35.886	55.631	50.294	47.270 ± 5.897	-	-	-	-
Recovery		61.966	73.046	69.076	68.029 ± 3.241	93.005	93.236	96.033	94.091 ± 0.973

[a] No feces were collected during this time period.

APPENDIX VI

SYNTHESIS OF ^{14}C-LABELED 4,4'-MDA

INTEROFFICE COMMUNICATION

MIDWEST RESEARCH INSTITUTE

September 20, 1984

To: Monaem El-hawari

From: David Chien and Bob Roth

Subject: [^{14}C]MDA Synthesis on Project No. 7901-A(21)/8201-A(21)

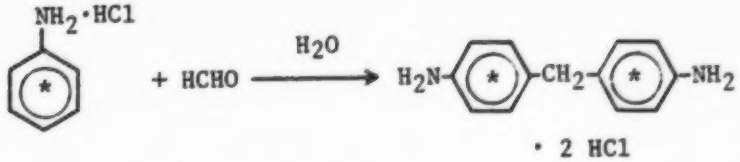
Attached is the synthesis procedure and analytical data for [^{14}C]MDA prepared on the subject project. A total of 3 samples were delivered: 1 x 90 mg (4.9 mCi) and 1 x 60 mg (3.28 mCi) of [^{14}C]MDA dihydrochloride (solid form) and 1 x 42.9 mg (3.2 mCi) of the free base dissolved in 3 mL of ethanol.

DC/BR:cs
Attachments

4,4'-Methylenedianiline [ring-U-^{14}C]

Experimental

Step 1 - 4,4'-Methylenedianiline [ring-U-^{14}C]

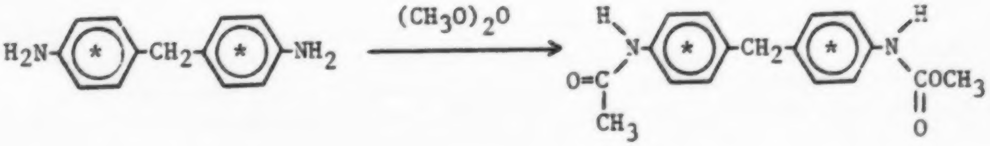


Literature Reference: J. T. Scanlan, J. Amer. Chem. Soc., 57, 890 (1935) [General synthesis], V. Henri, U.S. 4008275 [Purification].

MRI Reference: 83-25-73

To a solution of aniline hydrochloride [ring-U-^{14}C] (50.0 mCi, 3.70 mmole) in 2 mL of water and 7 µL of con. HCl was added 36% formaldehyde solution (171 µL, 2.03 mmole). The resulting solution was stirred at about 10°C for 10 min and then at 55-60°C for 6 hr. The reaction mixture was cooled to room temperature; and α-methylstyrene (194.6 mg, 1.65 mmole) and water (1 mL) were added. The resulting mixture was stirred at 94°C for 15 hr then cooled, basified with 5 mL of ammonia water and extracted with chloroform. The organic layer was dried (Na_2SO_4) and the solvent removed in vacuo to yield a dark brow oil (594 mg, 49 mCi). TLC silica gel 60, 90:25:4 benzene:dioxane:acetic acid, $R_f = 0.30$. The crude product was estimated to be about 40% pure.

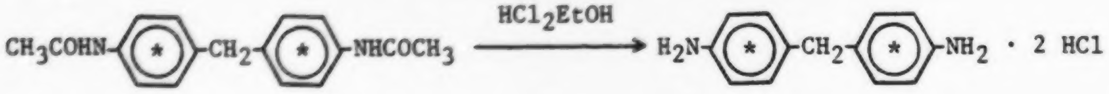
Step 2 - Purification via the N,N'-Diacetyl Derivative



MRI Reference: 83-25-76

The product from the previous step was dissolved in 5 mL of acetic anhydride and the resulting mixture was stirred at room temperature for 15 hr. The solvent was removed under vacuum to yield 750 mg of dark brown solid. Column chromatography on silica gel 60 (1 in. x 11 in.) eluted with chloroform/ ethanol 10:1 provided 4 mCi, 186 mg of light brown solid product TLC silica gel 60, 20:3 chloroform:ethanol $R_f = 0.34$. The product was estimated to be about 99% pure.

Step 3 - 4,4'-Methylenedianiline [ring-U-^{14}C]



MRI Reference: 81-25-78

The product from the previous step (186 mg, 14 mCi, 0.66 mmol) was dissolved in 20 mL of ethanol. Commercial 4,4'-dianiline methane (57.3 mg, 0.29 mmole) and conc. HCl (1.5 mL) were added, and the resulting mixture was stirred at 95°C for 16 hr. Solvent was removed in vacuo and the residue was washed with chloroform, benzene and 1.5 mL of cold ethanol to give 210 mg (11.46 mCi) of pure 4,4'-dianiline methane dihydrochloride (ring-U-^{14}C). A 60-mg (3.28 mCi) sample of dihydrochloride was treated with 5 mL of conc. NH_4OH and the free base extracted into CH_2Cl_2. The extract was dried (Na_2SO_4) and the solvent evaporated to give 42.9 mg (3.2 mCi) of the free base as a pink solid. This material was dissolved in 3 mL of ethanol and delivered along with 1 x 60 mg (3.28 mCi) and 1 x 90 mg (4.9 mCi) samples of the dihydrochloride salt to Monaem El-hawari on 6/8/84.

Analysis: The radiochemical purity of the hydrochloride salt was determined to be $\geqq$ 99% by TLC-radiochromatogram scans in two solvent systems. A more critical evaluation of purity was attempted using HPLC. Considerable effort was put into developing a suitable normal phase system using either a Waters µPoracil or Whatman Partisil 5 column and various mobile phases derived from combinations of acetonitrile, dioxane, tetrahydrofuran, methyl t-butyl ether, methylene chloride and hexane. The best system was found to be a Whatman Partisil 5 column in combination with a 40:60 (v/v) hexane/methyl t-butyl ether mobile phase. The initial analysis using this sytem on 6/19/84 indicated both a chemical and radiochemical purity > 98%. These results are presented in the following analytical summary.

On 7/23/84 the free base stock solution in ethanol was reanalyzed by the same HPLC method to check stability of the compound during storage. Due to changes in equilibration, the retention time of MDA was lengthened from 13 min to approximately 26 min. All MDA chromatograms including commercial and recrystallized commercial MDA's now contained a new peak, retention time about 23 min with a relative area of 5%. It was subsequently demonstrated that this 23-min peak was an artifact derived from t-butyl methyl ether induced decompositon of MDA.

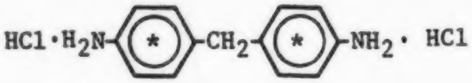
When a sample of commercial MDA was dissolved in t-butyl methyl ether and immediately injected, only the 23-min peak as observed in the chromatogam; the MDA peak at 26 min was completely absent.

ANALYTICAL DATA SUMMARY
MRI Project No. 7901-A(21)

COMPOUND: 4,4'-Dianiline methane dihydrochloride [ring-U-^{14}C]

LOT NO.: 83-25-78

STRUCTURE:



FORMULA: $C_{13}H_{16}N_2Cl_2$

AMOUNT: 150.0 mg (8.2 mCi)

SPECIFIC ACTIVITY: 14.8 mCi/mmole (gravimetric)

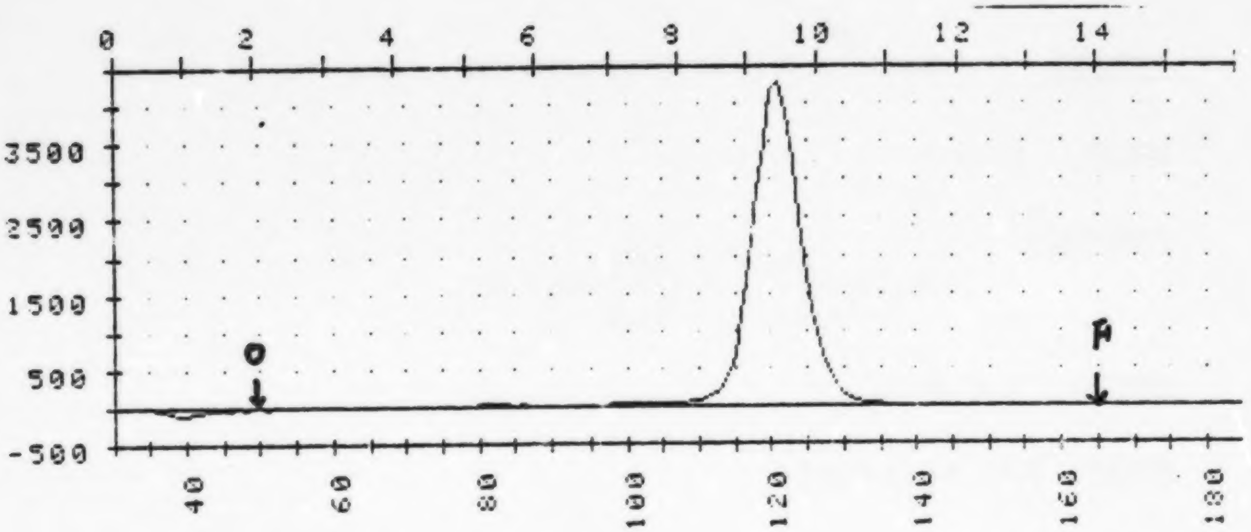
ESTIMATED PURITY: > 98% chemical and radiochemical

THIN-LAYER CHROMATOGRAPHY

Silica Gel 60, Chloroform:acetone:ammonium hydroxide, 1:8:1, $R_f = 0.90$
Benzene:acetone:ammonium hydroxide, 20:11:5, $R_f = 0.60$

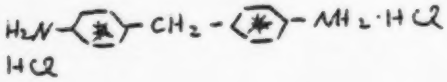
ATTACHED: TLC radiochromatogram (2)

BIOSCAN BID 100
TLC-Radiochromatogram Scan



Compound:

4,4'-Methylene dianiline hydrochloride
[phenyl-U-^{14}C]



$H_2N-\langle * \rangle-CH_2-\langle * \rangle-NH_2 \cdot HCl$
HCl

Lot No. MRI- 83-25-78

☒ Silica Gel 60

☐ Other:

Solvent System:

20 : 11 : 5 Benzene : Acetone
: Ammonium Hydroxide

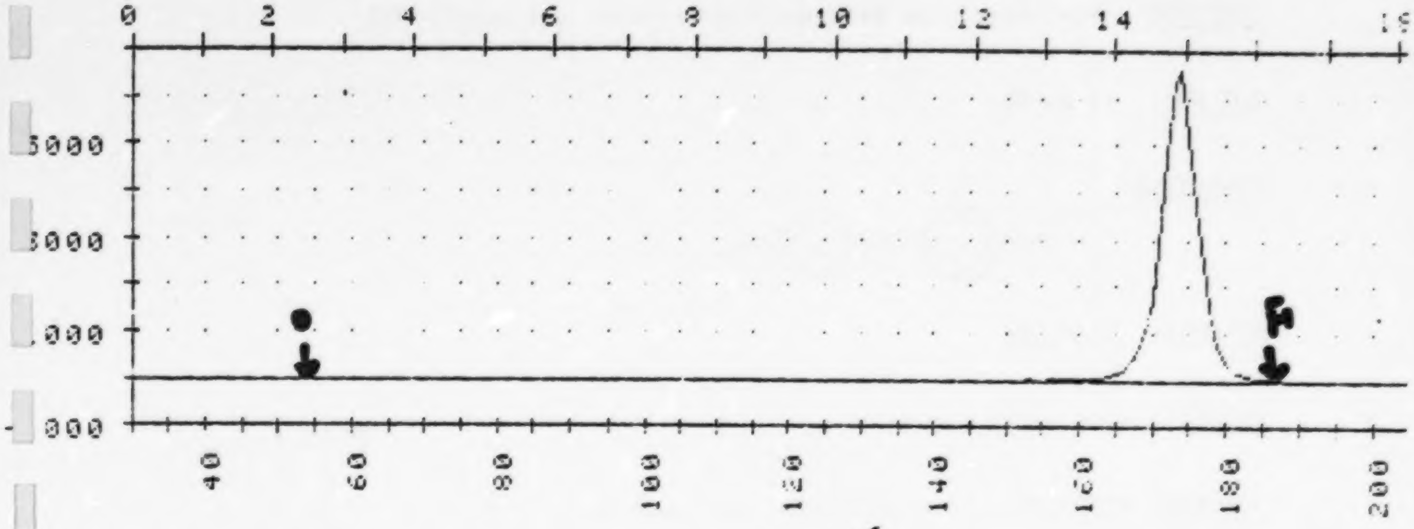
Integration List

```
Label:   METHYLENEDIANILINE HCL
Date:    31-005-84
Start:   00:24:38 Stop: 00:26:38
Accum:   00:02:00
Resolution: Normal
Total (30-224) = 34354

BACKGROUND REMOVED
```

```
Label: METHYLENEDIANILINE HCL
Chans:  107  thru 134
Sum:    34057
Centroid:  121.41 Chans
             94.88 MM
% of Tot(30-224):  99.14
```

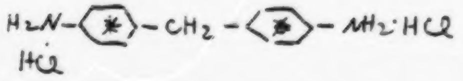
BIOSCAN BID 100
TLC-Radiochromatogram Scan



Compound:

4,4'-Methylene dianiline hydrochloride

[phenyl - U - ^{14}C]



Integration List

```
Label:   METHYLENEDIANILINE HCL
Date:    31-005-84
Start:   00:04:37 Stop  00:06:37
Accum:   00:02:00
Resolution: Normal
Total (30-224) = 39867

BACKGROUND REMOVED


Label: METHYLENEDIANILINE HCL
Chans:  162  thru 184
Sum:    39495
Centroid:  174.19 Chans
           149.65 MM
% of Tot(30-224):  99.07
```

Lot No. MRI- 83-25-78

☒ Silica Gel 60

☐ Other:

Solvent System:

1 : 8 : 1

Chloroform : Acetone : Ammonium Hydroxide

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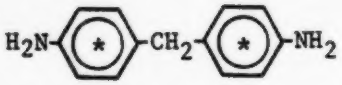
ANALYTICAL DATA SUMMARY
MRI Project No. 7901-A(21)

COMPOUND: 4,4'-Dianiline methane dihydrochloride [ring-U-^{14}C]

LOT NO.: 83-25-80

STRUCTURE:



FORMULA: $C_{13}H_{14}N_2$

AMOUNT: 42.9 mg (3.2 mCi)

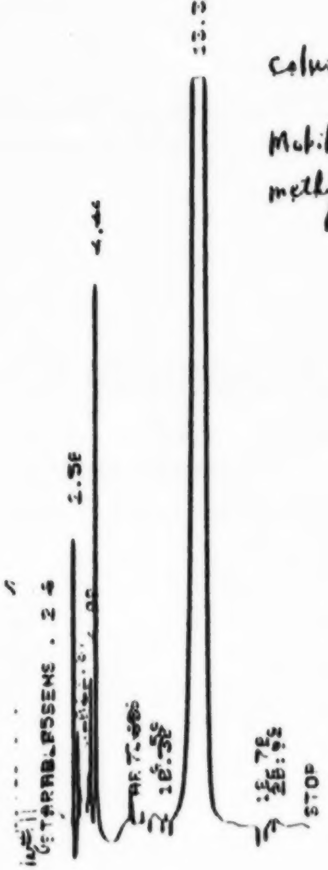
SPECIFIC ACTIVITY: 14.8 mCi/mmole (gravimetric)

ESTIMATED PURITY: > 98% chemical and radiochemical

HIGH PRESSURE LIQUID CHROMATOGRAPHY

Column: Whatman Partisil 5
Mobile Phase: Hexane/methyl-t-butyl ether, 40/60
Flow Rate: 1.3 mL/min
Detection: UV 254 and collection of 20-sec fractions with scintillation counting

ATTACHED: HPLC chromatogram (2)



```
HP RUN # 195
AREA %

RT        AREA        AREA %

 6.66      2858       0.148
13.32   1449088      96.756
16.78       661       0.045
19.69      4442       0.337
28.14     18576       0.728

DIL FACTOR: 1.0000 E- 0

ATTN 2^ 6
ATTN 2^ 5 0
```

Column : Whatman
Partisil 5
Mobile Phase : Hexane/
methyl-t-butyl ether 40/60

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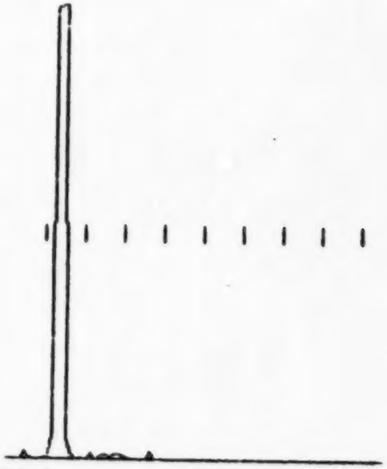
HPLC Analysis of 4,4'-Dianiline methane [ring-U-^{14}C), Lot No. 83-25-80
(Whatman Partisil 5, Hexane:Methyl-t-butyl ether, 40:60)
Sample RT = 13.32 min

```
14-C

 FILE: MDA 1
91 VIALS QUANTITATED
RECOVERED DPMS = 446931
PACKARD 4530 (3H/14C MODE)

57291 DPM
```



```
INTEGRATION OF RADIOACTIVITY

PEAKS ARE   0.2%
14-C FILE NAME MDA 1

INT ST      PK MAX     INT END INT DPM %OF TOTAL
12          14         19      477998  106.95%

TOTAL % IN INTEGRATION LIST= 106.95%
TOTAL DPMS RECOVERED = 446931
A BKG COUNT OF 750 IS CONSIDERED
```

HPLC Effluent Fraction Relative Radioactivity (6, 20-sec cuts) of 4,4'-Dianiline methane [ring-U-^{14}C), Lot No. 83-25-80